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<b>(54) Title:</b> DON-1 GENE AND POLYPEPTIDES AND USES THEREFOR		
<b>(57) Abstract</b>  The present invention relates to the identification and characterization of a novel gene called <i>don-1</i> related to epidermal growth factors (EGF) such as the neuregulins, and methods of preparing and using alternate splice forms of this gene to express new Don-1 polypeptides.		

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- 1 -

DON-1 GENE AND POLYPEPTIDES AND USES THEREFORBackground of the Invention

This invention relates to a new gene, called don-  
5 1, related to growth factors such as the neuregulins, and  
methods of preparing and using alternate splice forms of  
this gene to express new Don-1 polypeptides. The  
invention also relates to the use of these new genes and  
corresponding polypeptides.

10 The growth, differentiation, and survival of many  
cell types depends on the binding of protein ligands to  
specific cell surface receptors. Misregulation of this  
interaction has been implicated in a wide variety of  
tumors and developmental irregularities. For example,  
15 the epidermal growth factor receptor (EGFR) family of  
receptor-type tyrosine kinases are frequently  
overexpressed, mutated, or deleted in carcinomas of the  
breast, lung, ovary, brain, and gastrointestinal tract  
(Prigent et al., *Prog. Growth Factor Res.*, 4:1-24,  
20 1992). This family of receptors, which includes  
receptors referred to as EGFR, erbB2 (also called "neu"  
or HER2, the human homolog of erbB2), erbB3 (HER3), and  
erbB4 (HER4), respectively, may play an important role in  
the modulation of tumor growth and progression. In  
25 particular, it has been shown in several studies that  
overexpression of erbB2 in a variety of human  
adenocarcinomas, e.g., in breast and ovarian cancer,  
correlates with a poor prognosis (see, e.g., Slamon et  
al., *Science*, 235:177-182, 1987).

30 One group of ligands that bind to this family of  
receptors is referred to as the neuregulin family of  
ligands, which all share a common structural domain known  
as an EGF motif that contains six cysteines. This motif  
not only allows these ligands to bind to the receptors,  
35 but to mediate biological effects as well (Barbacci et  
al., *J. Biol. Chem.*, 270:9585-9589, 1995)). Although

- 2 -

there appear to be multiple ligands capable of binding to and activating members of the EGFR family, the growth factors that bind to and activate the other members of this receptor family, erbB2, erbB3, and erbB4, are less well characterized.

Neuregulins are also referred to as neu differentiation factors (NDF), glial growth factors (GGF), heregulins, and acetylcholine-receptor-inducing activity (ARIA) ligands, all of which are expressed as variant splice forms of a single gene. These different names reflect the diverse biological activities of the neuregulins *in vitro*, as glial cell mitogens, receptor binding proteins, mammary differentiation factors, and muscle trophic factors.

Each of the neuregulin glycoproteins has been shown to activate one or more of the receptors erbB2, erbB3, and erbB4 (for a review, see Ben-Baruch et al., *Proc. Soc. Exp. Biol. Med.*, 206:221-227, 1994). These factors were first purified on the basis of their ability to activate, i.e., cause phosphorylation of, the erbB2 receptor, although it has been shown subsequently that these factors do not bind erbB2 directly (Tzahar et al., *J. Biol. Chem.*, 269:25226-25233, 1994). In addition, it has been shown that NDF causes the differentiation of human mammary tumor cells (Peles et al., *Cell*, 69:559-572, 1992).

#### Summary of the Invention

The present invention relates to the identification and characterization of a new gene, referred to as *don-1*, and alternate splice variants of *don-1*, which are related to the neuregulin gene family. The invention also relates to the polypeptides encoded by *don-1*. *Don-1* mRNA transcripts were expressed in various tissues including murine brain, spleen, and lung, and



- 3 -

human fetal brain and fetal lung. No Don-1 transcripts were detected in normal adult human tissues; however, Don-1 transcripts were detected in several human carcinoma cells. In each case, message sizes were about  
5 3.0 kb and 4.4 kb (human) and 4.0 kb (murine).

Both murine and human cDNAs corresponding to various splice variants of *don-1* have been cloned. A murine cDNA corresponding to a first splice variant of this gene is represented by SEQ ID NO:1, and the amino  
10 acid sequence of the polypeptide it encodes is represented by SEQ ID NO:2, which is a membrane-bound polypeptide approximately 605 amino acids in length (Fig. 1). A second murine cDNA corresponding to a second splice variant of the *don-1* gene is represented by SEQ ID  
15 NO:3, and the amino acid sequence of the polypeptide it encodes is represented by SEQ ID NO:4, which is a secreted polypeptide about 181 amino acids in length (Fig. 2).

A human cDNA corresponding to a first splice  
20 variant of the human *don-1* gene is represented by SEQ ID NO:5, and the amino acid sequence of the polypeptide it encodes is represented by SEQ ID NO:6, which is a membrane-bound polypeptide approximately 407 amino acids in length (Fig. 3). A second human cDNA corresponding to  
25 a second splice variant of the human *don-1* gene is represented by SEQ ID NO:7, and the amino acid sequence of the polypeptide it encodes is represented by SEQ ID NO:8, which is a membrane-bound polypeptide of about 469 amino acids in length (Fig. 4).

30 A third human cDNA corresponding to a third splice variant of the human *don-1* gene was isolated by further screening of a human fetal lung library. This sequence had an extended sequence compared to the first two clones, and included a termination codon. This sequence  
35 is represented by SEQ ID NO:31, and the amino acid

- 4 -

sequence of the polypeptide it encodes is represented by SEQ ID NO:32, which is a membrane-bound polypeptide of about 647 amino acids in length (Fig. 7). This sequence appears to be an extended version of the second splice variant (SEQ ID NO:8), although three amino acids differ at the 3' end of SEQ ID NO:32. This third splice variant extends a further 178 amino acids compared to the second human splice variant, and is 94% homologous to murine Don-1 (SEQ ID NO:2) over this region.

10 In addition, the invention relates to methods of obtaining additional novel ligands that activate some or all members of the EGF receptor family of receptor-type tyrosine kinases, and methods of treating and diagnosing cell proliferative diseases.

15 In general, the invention features an isolated nucleic acid which encodes a mammalian Don-1 polypeptide, e.g., a polypeptide encoded by any splice variant of a *don-1* gene. For example, the nucleic acid can include all or a portion of the nucleotide sequence of, e.g.,  
20 Fig. 1, SEQ ID NO:1 (murine), Fig. 2, SEQ ID NO:3 (murine), Fig. 3, SEQ ID NO:5 (human), Fig. 4, SEQ ID NO:7 (human), Fig. 7, SEQ ID NO:31 (human), the sequence encoding the epidermal growth factor (EGF) domain of Don-1 having SEQ ID NO:11, or the extracellular domain of  
25 Don-1.

The term "nucleic acid" encompasses both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. The nucleic acid may be double-stranded or single-stranded. Where single-  
30 stranded, the nucleic acid may be a sense strand or an antisense strand.

By "isolated nucleic acid" is meant a DNA or RNA that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous  
35 (one on the 5' end and one on the 3' end) in the

- 5 -

naturally occurring genome of the organism from which it is derived. Thus, in one embodiment, an isolated nucleic acid includes some or all of the 5' non-coding (e.g., promoter) sequences which are immediately contiguous to the coding sequence. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence. The term "isolated" as used herein also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an "isolated nucleic acid" is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state.

A nucleic acid sequence that is "substantially identical" to a *don-1* nucleotide sequence is at least 80% or 85%, preferably 90%, and more preferably 95% or more (e.g. 99%) identical to the nucleotide sequence of the human *don-1* cDNA of SEQ ID NO:5, NO:7, or NO:31, or the murine *don-1* cDNA of SEQ ID NO:1 or NO:3. For purposes of comparison of nucleic acids, the length of the reference nucleic acid sequence will generally be at least 40 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 to 110, or more nucleotides.

Sequence identity can be measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of

- 6 -

Wisconsin Biotechnology Center, 1710 University Avenue,  
Madison, WI 53705).

The invention also encompasses nucleic acid  
sequences that encode forms of Don-1 in which naturally  
5 occurring amino acid sequences are altered or deleted.

The invention also features isolated nucleic acid  
sequences that encode one or more portions or domains of  
Don-1, including but not limited to the Ig domain, the TM  
domain, the extracellular domain, the cytoplasmic domain,  
10 and various functional domains of Don-1, such as the EGF  
domain. The nucleic acids also include those of the *don-1*  
gene contained in A.T.C.C. deposit numbers 98096,  
98097, or 98098.

Preferred nucleic acids encode polypeptides that  
15 are soluble under normal physiological conditions. Also  
within the invention are nucleic acids encoding fusion  
proteins in which a portion of Don-1 (e.g., one or more  
domains) is fused to an unrelated protein or polypeptide  
(e.g., a marker polypeptide or a fusion partner) to  
20 create a fusion protein. For example, the polypeptide  
can be fused to a hexa-histidine tag to facilitate  
purification of bacterially expressed protein, or to a  
hemagglutinin tag to facilitate purification of protein  
expressed in eukaryotic cells.

25 The fusion partner can be, for example, a  
polypeptide which facilitates secretion, e.g., a  
secretory sequence. Such a fused protein is typically  
referred to as a preprotein. The secretory sequence can  
be cleaved by the host cell to form the mature protein.  
30 Also within the invention are nucleic acids that encode  
mature Don-1 fused to a polypeptide sequence to produce  
an inactive proprotein. Proproteins can be converted  
into the active form of the protein by removal of the  
inactivating sequence.

- 7 -

The nucleic acids further include nucleic acids that hybridize, e.g., under stringent hybridization conditions (as defined herein), to all or a portion (e.g., the TM or EGF domains) of the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 31, or its complement, or to the nucleotide sequence of the *don-1* gene contained in A.T.C.C. deposit 98096, 98097, or 98098, e.g., nucleic acids that encode polypeptides that activates receptor-type tyrosine kinases that have a molecular weight of about 185 kDa.

The hybridizing portion of the hybridizing nucleic acids are preferably 20, 30, 50, or 70 bases long. Preferably, the hybridizing portion of the hybridizing nucleic acid is 80%, more preferably 95%, or even 98% identical to the sequence of a portion or all of a nucleic acid encoding a Don-1 polypeptide. Hybridizing nucleic acids of the type described above can be used as a cloning probe, a primer (e.g., a PCR primer), or a diagnostic probe. Preferred hybridizing nucleic acids encode a polypeptide having some or all of the biological activities possessed by a naturally-occurring Don-1 polypeptide, e.g., as determined in the p185 assay described below.

Hybridizing nucleic acids can be additional splice variants of the *don-1* gene. Thus, they may encode a protein which is shorter or longer than the different forms of Don-1 described herein. Hybridizing nucleic acids may also encode proteins that are related to Don-1 (e.g, proteins encoded by genes which include a portion having a relatively high degree of identity to the *don-1* gene described herein).

In another embodiment, the invention features cells, e.g., transformed host cells, harboring a nucleic acid encompassed by the invention. By "transformed cell" is meant a cell into which (or into an ancestor of which)

- 8 -

has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding a Don-1 polypeptide.

The invention also features vectors and plasmids that include a nucleic acid of the invention which is  
5 operably linked to a transcription and/or translation sequence to enable expression, e.g., expression vectors. By "operably linked" is meant that a selected nucleic acid, e.g., a DNA molecule encoding a Don-1 polypeptide, is positioned adjacent to one or more sequence elements,  
10 e.g., a promoter, which direct transcription and/or translation of the sequence such that the sequence elements can control transcription and/or translation of the selected nucleic acid.

The invention also features purified or isolated  
15 Don-1 polypeptides. As used herein, both "protein" and "polypeptide" mean any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation). Thus, the term "Don-1 polypeptide" (or Don-1) includes full-length, naturally  
20 occurring Don-1 protein, as well as recombinantly or synthetically produced polypeptides that correspond to a full-length, naturally occurring Don-1 protein or to particular domains or portions of a naturally occurring protein.

25 By a "purified" or "isolated" compound is meant a composition which is at least 60% by weight (dry weight) the compound of interest, e.g., a Don-1 polypeptide or antibody. Preferably the preparation is at least 75%, more preferably at least 90%, and most preferably at  
30 least 99%, by weight the compound of interest. Purity can be measured by any appropriate standard method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

Preferred Don-1 polypeptides include a sequence  
35 substantially identical to all or a portion of a

- 9 -

naturally occurring Don-1 polypeptide, e.g., including all or a portion of the human sequence shown in Fig. 3 (SEQ ID NO:6), Fig. 4 (SEQ ID NO:8), or Fig. 7 (SEQ ID NO:32), or the murine sequence shown in Fig. 1 (SEQ ID NO:2) or Fig. 3 (SEQ ID NO:6). Polypeptides "substantially identical" to the Don-1 polypeptide sequences described herein have an amino acid sequence that is at least 80% or 85%, preferably 90%, and more preferably 95% or more (e.g. 99%) identical to the amino acid sequence of the Don-1 polypeptides of SEQ ID NOs:2, 4, 6, or 8. For purposes of comparison, the length of the reference Don-1 polypeptide sequence will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids.

In the case of polypeptide sequences which are less than 100% identical to a reference sequence, the non-identical positions are preferably, but not necessarily, conservative substitutions for the reference sequence. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine.

Where a particular polypeptide is said to have a specific percent identity to a reference polypeptide of a defined length, the percent identity is relative to the reference peptide. Thus, a peptide that is 50% identical to a reference polypeptide that is 100 amino acids long can be a 50 amino acid polypeptide that is completely identical to a 50 amino acid long portion of the reference polypeptide. It also might be a 100 amino acid long polypeptide which is 50% identical to the reference

- 10 -

polypeptide over its entire length. Of course, many other polypeptides will meet the same criteria.

The polypeptides of the invention include, but are not limited to: recombinant polypeptides, natural  
5 polypeptides, and synthetic polypeptides as well as polypeptides, which are preproteins or proproteins.

Polypeptides identical or substantially identical to one or more domains of human, murine, or other mammalian Don-1, e.g., the EGF domain (e.g., SEQ ID  
10 NO:11) (about amino acid 142 to about amino acid 178 of human Don-1 cDNA SEQ ID NOs:8 and 32, or amino acids 104 to 140 of human Don-1 cDNA SEQ ID NO:6 described herein), or the transmembrane (TM) domain (e.g., SEQ ID  
NO:20) (about amino acid 203 to about amino acid 225 of  
15 human Don-1 cDNA SEQ ID NOs:8 and 32, or amino acids 173 to 195 of human Don-1 cDNA SEQ ID NO:6 described herein), are also within the scope of the invention.

Polypeptides encoded by the *don-1* gene contained in A.T.C.C. deposit 98096, 98097, or 98098 are also  
20 included within the invention.

Preferred polypeptides are those which are soluble under normal physiological conditions. Also within the invention are soluble fusion proteins in which a full-length form of Don-1 or a portion (e.g., one or more  
25 domains) thereof is fused to an unrelated protein or polypeptide (i.e., a fusion partner) to create a fusion protein.

The invention also features isolated polypeptides (and the nucleic acids that encode these polypeptides)  
30 that include a first portion and a second portion; the first portion includes a Don-1 polypeptide, e.g., the epidermal growth factor (EGF) domain of Don-1, and the second portion includes an immunoglobulin constant (Fc) region or a detectable marker.



- 11 -

In addition, the invention features a pharmaceutical composition which includes a Don-1 polypeptide and a physiologically acceptable or inert carrier, such as saline.

5           The invention also features purified or isolated antibodies that specifically bind to a Don-1 polypeptide, or a specific region or domain of a naturally occurring Don-1 protein. By "specifically binds" is meant an antibody that recognizes and binds to a particular  
10 antigen, e.g., a Don-1 polypeptide, but which does not substantially recognize and bind to other molecules in a sample, e.g., a biological sample, which naturally includes Don-1. In a preferred embodiment the antibody is a monoclonal antibody.

15           The invention also features antagonists and agonists of Don-1. Antagonists can inhibit one or more of the functions of Don-1. Suitable antagonists include large or small molecules, antibodies to Don-1, and Don-1 polypeptides which compete with a native form of Don-1.  
20 Agonists of Don-1 enhance or facilitate one or more of the functions of Don-1. Suitable agonists include, for example, large or small molecules and anti-idiotypic antibodies that mimic the biological effects of Don-1.

          Also within the invention are nucleic acid  
25 molecules that can be used to interfere with Don-1 expression, e.g., antisense molecules and ribozymes.

          In another aspect, the invention features a method for detecting a Don-1 polypeptide. This method includes: obtaining a biological sample; contacting the sample with  
30 an antibody, that specifically binds a Don-1 polypeptide, under conditions that allow the formation of Don-1-antibody complexes; and detecting the complexes, if any, as an indication of the presence of Don-1 in the biological sample.

- 12 -

In another aspect, the invention features a method for stimulating proliferation of a cell, by administering to the cell an amount of a Don-1 polypeptide effective to stimulate proliferation of the cell. The invention also  
5 features a method for decreasing proliferation of a cell, by administering to the cell an amount of a Don-1 polypeptide inhibitor effective to decrease proliferation of the cell. This method can be used to treat tumors, e.g., adenocarcinomas, caused by the over-proliferation  
10 of cells in a patient. Preferably the inhibitor is an antibody which selectively binds to Don-1.

In another embodiment, the invention features a method of obtaining a splice variant cDNA of the *don-1* gene. The method includes the steps of obtaining a  
15 labeled probe comprising an isolated nucleic acid that encodes all or a portion of the epidermal growth factor (EGF) domain of Don-1, e.g., having the amino acid sequence of SEQ ID NO:11; screening a nucleic acid fragment library with the labeled probe under conditions  
20 that allow hybridization of the probe to nucleic acid fragments in the library to form nucleic acid duplexes, isolating labeled duplexes, if any; and preparing a full-length cDNA from the fragments in any labeled duplex to obtain a splice variant cDNA of the *don-1* gene.

25 The invention further features a method of obtaining a gene related to the *don-1* gene, by obtaining a labeled probe comprising an isolated nucleic acid that encodes all or a portion of the transmembrane (TM) domain of Don-1, e.g., having the amino acid sequence of SEQ ID  
30 NO:20; screening a nucleic acid fragment library with the labeled probe under conditions that allow hybridization of the probe to nucleic acid fragments in the library to form nucleic acid duplexes; isolating labeled duplexes, if any; and preparing a full-length gene sequence from

- 13 -

the nucleic acid fragments in any labeled duplex to obtain a gene related to the *don-1* gene.

The invention also features a purified protein that functionally interacts with Don-1, and a nucleic acid that encodes a protein that functionally interacts with Don-1.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described herein. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting

Other features and advantages of the invention will be apparent from the following detailed descriptions, and from the claims.

#### Brief Description of the Drawings

Fig. 1 is a representation of the nucleic acid (SEQ ID NO:1) of a murine cDNA corresponding to a membrane-bound splice variant of the *don-1* gene, and the amino acid sequence (SEQ ID NO:2) it encodes.

Fig. 2 is a representation of the nucleic acid (SEQ ID NO:3) of a second murine cDNA corresponding to a secreted splice variant of the *don-1* gene, and the amino acid sequence (SEQ ID NO:4) it encodes.

Fig. 3 is a representation of the nucleic acid (SEQ ID NO:5) of a human cDNA corresponding to a

- 14 -

membrane-bound splice variant of the human *don-1* gene, and the amino acid sequence (SEQ ID NO:6) it encodes.

Fig. 4 is a representation of the nucleic acid (SEQ ID NO:7) of a human cDNA corresponding to a second  
5 splice variant of the human *don-1* gene, and the amino acid sequence (SEQ ID NO:8) it encodes.

Fig. 5 is a multi-sequence alignment of the amino acid SEQ ID NOs:2, 4, 6, and 8 of Figs. 1 to 4, as well as the amino acid sequence of rat neu differentiation  
10 factor (NDF) (Genbank Accession No. A38220; SEQ ID NO:9) and human heregulin- $\beta$  (Genbank Accession No. B43273; SEQ ID NO:10). In this figure, an asterisk above the aligned sequences indicates the location of conserved cysteines in the EGF domain. The transmembrane domains are boxed.

15 Fig. 6 is a representation of a sequence alignment of the EGF domain of Don-1 (SEQ ID NO:11) with the growth factor domains of members of the neuregulin/heregulin family and human heparin binding-EGF (hb-EGF). The domain is bounded by cysteines, and contains a total of  
20 six conserved cysteines. Fig. 6 shows additional amino acids upstream and downstream of the EGF domain. Amino acid sequences correspond to a Don-1 EGF polypeptide (SEQ ID NO:11), human heregulin- $\alpha$  (Genbank Accession No. A43273, SEQ ID NO:12), rat NDF (Genbank Accession No.  
25 A38220; SEQ ID NO:13), human heregulin- $\beta$ 1 (Genbank Accession No. A43273; SEQ ID NO:14), chicken ARIA (Genbank Accession No. A45769; SEQ ID NO:15); human heparin binding-EGF (Genbank Accession No. A38432; SEQ ID NO:16); human EGF (Genbank Accession No. P01133; SEQ ID  
30 NO:17); human amphiregulin (Genbank Accession No. 179040; SEQ ID NO:18); and human TGF- $\alpha$  (Genbank Accession No. 339546; SEQ ID NO:19).

Fig. 7 is a representation of the nucleic acid (SEQ ID NO:31) of a human cDNA corresponding to a third

- 15 -

splice variant of the human *don-1* gene, and the amino acid sequence (SEQ ID NO:32) it encodes.

#### Detailed Description

Don-1 polypeptides, described here for the first time, are a family of novel glycoprotein ligands related to epidermal growth factors such as the neuregulins. The different Don-1 polypeptides are encoded by different splice variants of the *don-1* gene. Don-1 plays a role in proliferation of carcinomas including adenocarcinoma, myeloma, glioma, melanomas, as well as in cell differentiation, proliferation, and survival.

Don-1 polypeptides have a mosaic grouping of functional domains similar to those found in neuregulins (Wen et al., *Cell*, **69**, 559-572, 1992). For example, similar to NDF, both secreted and membrane-bound forms of Don-1 polypeptides include an EGF domain, which enables these ligands to bind to EGF receptors, and to mediate biological effects. As described herein, the EGF domain can also be used to obtain additional splice variants of the *don-1* gene.

Also like NDF, membrane-bound forms of Don-1 (SEQ ID NOs:2, 6, 8, and 32) contain a recognized Ig domain, a transmembrane (TM) domain (VLTITGICVALLVVGIVCVVAYC, SEQ ID NO:20), and a cytoplasmic domain. The Ig domain should be important in protein-protein interactions. As described herein, the TM domain can be used to obtain additional new genes related to the *don-1* gene. A secreted form of murine Don-1 (SEQ ID NO:4) is a variant splice form that lacks the transmembrane sequence. These domains are described in detail below.

As shown in Fig. 5, comparison of a sequence of a human cDNA of Don-1 (SEQ ID NO:8) isolated from human fetal brain, revealed that the EGF domain (about amino acid 142 to about amino acid 178) is 100% identical to

- 16 -

the EGF domain in the mouse Don-1 amino acid sequence of SEQ ID NO:2 (about amino acids 104 to 140). In addition, the TM domains (boxed in Fig. 5) appear to be highly conserved between mouse and human Don-1 (identical; SEQ ID NO:20), and between Don-1, NDF, and heregulin (2 differences of 23 amino acids). The generic TM domain sequence is VLTITGICX<sub>1</sub>ALLVVGIX<sub>2</sub>CVVAYC (SEQ ID NO:21), where X<sub>1</sub> is I or V, and X<sub>2</sub> is M or V.

The two neighboring basic amino acids adjacent the transmembrane region (amino acids Lys-171 and Arg-172 in the human SEQ ID NO:6; amino acids Lys-201 and Arg-202 in the human SEQ ID NOS:8 and 32; amino acids Lys-163 and Arg-164 in the murine form SEQ ID NO:2) provide for the possibility of processing these proteins with proteolytic enzymes to detach them from the cell membrane.

Fig. 5 shows the primary structure of both murine and human forms of Don-1 (SEQ ID NOS:2, 4, 6, and 8), as well as the primary structures of rat NDF (SEQ ID NO:9), human heregulin- $\beta$  (SEQ ID NO:10). As can be seen from this figure, these sequences have highly conserved Ig, EGF (extracellular) and TM domains. Further, there is high homology in the cytoplasmic domains.

Expression of Don-1 in human tissues appeared to be restricted to fetal brain and lung tissues. No Don-1 transcripts were detected in normal adult human tissues using a murine Don-1 cDNA as a probe. However, Don-1 transcripts were detected in a human colon adenocarcinoma cell line SW480 and in a human melanoma cell line G361. In these tissues there were two major Don-1 transcripts of about 4.4 kb and about 3 kb each.

Overall, the human Don-1 cDNA of SEQ ID NO:8 described herein is 95% identical and 98% similar (based on conservative substitutions) at the amino acid level to the murine Don-1 cDNA of SEQ ID NO:2 described herein. The highest homology between the two forms is found in

- 17 -

the EGF and transmembrane domains, suggesting that both domains have important functional roles. High homology between the two forms is also found in the Ig and cytoplasmic domains.

#### 5 Don-1 Proteins and Polypeptides

Don-1 proteins and polypeptides and Don-1 fusion proteins can be prepared for a wide range of uses including, but not limited to, generation of antibodies, preparation of reagents for diagnostic assays,  
10 identification of other molecules involved in neoplastic and proliferation (particularly adenocarcinoma), preparation of reagents for use in screening assays for neoplasm modulators, and preparation of therapeutic agents for treatment of tumor-related disorders.

15 The *don-1* gene was originally isolated from a screen of a murine choroid plexus cDNA library. Further screening of other murine and human tissue sources yielded three additional clones of this gene, all representing different splice variants. Based on these  
20 cDNA sequences, the *don-1* gene can also be obtained by chemical synthesis using one of the methods described in Engels et al. (*Agnew. Chem. Int. Ed. Engl.*, 28:716-734, 1989). These methods include triester, phosphite, phosphoramidite and H-Phosphonate methods, PCR and other  
25 autoprimer methods, and oligonucleotide syntheses on solid supports. These methods may be used if the entire nucleic acid sequence of the gene is known, or the sequence of the nucleic acid complementary to the coding strand is available, or alternatively, if the target  
30 amino acid sequence is known, one may infer potential nucleic acid sequences using known and preferred coding residues for each amino acid residue.

In particular, Fig. 1 shows the cDNA of one murine splice variant of *don-1* (SEQ ID NO:1), which encodes a

- 18 -

predicted protein of about 605 amino acids (SEQ ID NO:2). This clone was isolated from a murine lung cDNA library. The Ig domain begins at a cysteine at about location 16 and extends to a cysteine at about location 70, and  
5 should be important in protein-protein interactions. The EGF domain (SEQ ID NO:11), which is predicted to contain the active part of the protein, begins at a cysteine at about amino acid location 104 and extends to a cysteine at about amino acid location 140 in this cDNA.

10 The spacing of the 6 cysteine residues and an important glycine residue (amino acid 137) in the EGF domain, are conserved between Don-1 and EGF, although homology over this region reveals that Don-1 is more similar to NDF (47% identity) than EGF (35% identity).  
15 In general, the EGF domain of Don-1 related polypeptides requires the following formula: the first C, followed by 7 amino acids; the second C, followed by 4 or 5 amino acids; the third C, followed by 10-13 amino acids; the fourth C, followed by 1 amino acid; the fifth C, followed  
20 by 8 amino acids; and then the sixth C.

The EGF domain of Don-1 (CNETAKSYCVNGGVCYYIEGINQL-SCKCPNGFFGQRC, SEQ ID NO:11) is identical in all five splice variants, both murine and human. Thus, probes designed based on the nucleotide region encoding this EGF  
25 domain can be used, as described herein, to obtain, in humans, mice, and other animals, additional splice variant cDNAs of the *don-1* gene.

The murine Don-1 polypeptide of Fig. 1 also includes a TM domain of approximately 23 amino acids  
30 extending from about amino acid location 165 to about amino acid location 187. Immediately prior to the TM domain are two basic residues (amino acids 163 and 164) that should function as a proteolytic cleavage site. This would result in the release of soluble ligand from  
35 the cell membrane. The cytoplasmic domain of Don-1



- 19 -

extends from about amino acid 183 to about amino acid 605.

The Don-1 TM domain (VLTITGICVALLVVGIVCVVAYC, SEQ ID NO:20), like the EGF domain, is also highly conserved in the murine and human membrane-bound splice variants of Don-1 that include this domain (murine SEQ ID NO:4 does not). In fact, the TM domain is identical in both human splice variants and the membrane-bound form of the murine splice variants. As shown in Fig. 5, this Don-1 TM domain is also highly conserved in other, related proteins, such as rat NDF, and human heregulin- $\beta$ . Thus, probes designed based on the nucleotide region encoding this TM domain can be used as described herein to obtain, in humans, mice, and other animals, additional genes related to the *don-1* gene.

Fig. 2 shows a second murine cDNA that corresponds to another splice variant of murine *don-1* (SEQ ID NO:3), which encodes a Don-1 polypeptide of 181 amino acids (SEQ ID NO:4). To obtain the nucleotide and amino acids sequences in Fig. 2, a 1.4kb cDNA that contained an open reading frame of 139 amino acids was isolated from a mouse choroid plexus library. This partial clone contained no 5' ATG initiation codon and terminated after the EGF domain. This original clone was then used as a probe to isolate other mouse and human splice variants. The other murine splice variant, SEQ ID NO:1 (Fig. 1), represents a longer, transmembrane-bound version of the original clone. Based on the high homology between the two mouse clones over the Ig and EGF domains, the chimeric clone of Fig. 2 was constructed and designated as the murine Don-1 cDNA of SEQ ID NO:3. This cDNA encompasses the nucleotide sequence encoding the first 42 amino acids of murine Don-1 SEQ ID NO:2, and the remaining 139 amino acids of the original murine Don-1

- 20 -

clone. This resulting chimera is 181 amino acids in length.

This splice variant does not contain a TM domain, and is thus a secreted protein. The structure of this  
5 second splice variant is identical to the polypeptide of SEQ ID NO:2 from amino acid 1 to amino acid 155. Thus, the EGF domain (SEQ ID NO:11), which is predicted to contain the biologically active part of the protein, begins at about amino acid location 104 and extends to  
10 amino acid location 140 in this cDNA.

Fig. 3 shows a cDNA of a human splice variant of the *don-1* gene (SEQ ID NO:5), which encodes a polypeptide of about 407 amino acids in length (SEQ ID NO:6). This clone was isolated from a human fetal lung cDNA library.  
15 This polypeptide includes an apparent Ig domain extending from a cysteine at about location 16 to a cysteine at about location 70; an EGF domain extending from a cysteine at about location 104 to a cysteine at about amino acid location 140; a transmembrane domain from  
20 about amino acid 173 to about amino acid 195; and a cytoplasmic domain of approximately 211 amino acids extending from about amino acid 196 to about amino acid 407. In addition, this splice variant includes an extra 8 amino acids in the juxtamembrane region (at locations  
25 157 to 164) compared to the other three splice variants.

Fig. 4 shows a second human cDNA corresponding to another splice variant of human *don-1* (SEQ ID NO:7), which encodes a polypeptide of about 469 amino acids in length (SEQ ID NO:8). This second human clone was also  
30 isolated from a human fetal lung cDNA library. This polypeptide includes an apparent Ig domain extending from a cysteine at about location 54 to a cysteine at about location 108; an EGF domain extending from about amino acid location 142 to about amino acid location 178; a  
35 transmembrane domain from about amino acid location 203

- 21 -

to about amino acid location 225; and a cytoplasmic domain of approximately 243 amino acids extending from about amino acid 226 to amino acid 469.

Fig. 7 shows a third human cDNA corresponding to a  
5 third splice variant of the human *don-1* gene (SEQ ID NO:31), which encodes a polypeptide of about 647 amino acids in length (SEQ ID NO:32). This third human clone was also isolated from a human fetal lung cDNA library. This polypeptide includes an apparent Ig domain extending  
10 from a cysteine at about location 54 to a cysteine at about location 108; an EGF domain extending from about amino acid location 142 to about amino acid location 178; a transmembrane domain from about amino acid location 203 to about amino acid location 225; and a cytoplasmic  
15 domain of approximately 421 amino acids extending from about amino acid 226 to amino acid 647 (which is the end of the polypeptide in view of the termination codon).

The invention encompasses, but is not limited to, Don-1 proteins and polypeptides that are functionally  
20 related to Don-1 encoded by the nucleotide sequences of Fig. 1 (murine SEQ ID NO:1), Fig. 2 (murine SEQ ID NO:3), Fig. 3 (human SEQ ID NO:5), Fig. 4 (human SEQ ID NO:7), and Fig. 7 (human SEQ ID NO:31). Functionally related proteins and polypeptides include any protein or  
25 polypeptide sharing a functional characteristic with Don-1, e.g., the ability to affect cell differentiation, proliferation, or survival, and those that are active in the p185 assay described herein.

Such functionally related Don-1 polypeptides  
30 include, but are not limited to, polypeptides with additions or substitutions of amino acid residues within the amino acid sequence encoded by the *don-1* cDNA sequences described herein which result in a silent change, thus producing a functionally equivalent gene  
35 product. Amino acid substitutions may be made on the

- 22 -

basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. The function of the new polypeptide can then be tested in the p185 assay

5 described herein.

For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, 10 tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

While random mutations can be made to *don-1* DNA 15 (using random mutagenesis techniques well known in the art) and the resulting mutant Don-1 proteins can be tested for activity, site-directed mutations of the *don-1* coding sequence can be engineered (using site-directed mutagenesis techniques well known to those skilled in the 20 art) to generate mutant Don-1 polypeptides with increased function, e.g., greater modulation of cell proliferation, differentiation or survival, or decreased function, e.g., down-modulation of cell proliferation, differentiation, or survival.

25 To design functionally related and/or variant Don-1 polypeptides, it is useful to distinguish between conserved positions and variable positions. Fig. 5 shows an alignment between the amino acid sequences of the human and murine Don-1 polypeptides. This alignment can 30 be used to determine the conserved and variable amino acid positions. To preserve Don-1 function, it is preferable that conserved residues are not altered. Moreover, alteration of non-conserved residues are preferably conservative alterations, e.g., a basic amino 35 acid is replaced by a different basic amino acid. To

- 23 -

produce altered function variants, it is preferable to make non-conservative changes at variable and/or conserved positions. Deletions at conserved and variable positions can also be used to create altered function  
5 variants.

Other mutations to the *don-1* coding sequence can be made to generate Don-1 polypeptides that are better suited for expression, scale up, etc. in a selected host cell. For example, N-linked glycosylation sites can be  
10 altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first  
15 or third amino acid positions of any one or more of the glycosylation recognition sequences which occur (in N-X-S or N-X-T), and/or an amino acid deletion at the second position of any one or more of such recognition sequences, will prevent glycosylation at the modified  
20 tripeptide sequence. (See , e.g., Miyajima et al., *EMBO J.*, 5:1193, 1986).

Preferred Don-1 polypeptides are those polypeptides, or variants thereof, that activate receptor-type tyrosine kinases which have a molecular  
25 weight of 185 kDa, which includes p185 (erbB2). Activating Don-1 polypeptides can be determined by a standard p185 assay as described herein. Briefly, the activity of the EGF domain of Don-1 was ascertained by testing the ability of an EGF domain-containing fusion  
30 polypeptide to phosphorylate a 185 kDa protein in the breast adenocarcinoma cell line MDA-MB453. Serum-starved cells were treated with EGF, NDF, conditioned media from mock-transfected or Don-1 EFG-transfected 293Ebna cells as described below. Analysis of phosphorylated proteins  
35 by Western blotting revealed that Don-1 EGF induced

- 24 -

phosphorylation of p185 at a level comparable to saturating amounts of NDF, which represented an approximate ten-fold increase in phosphorylation over uninduced cells. This result demonstrates that the EGF domain of Don-1 binds and activates a known member of the EGFR family, p185.

Preferred Don-1 polypeptides and variants have 20%, 50%, 75%, 90%, or even 100% or more of the activity of the human form of Don-1 (SEQ ID NOS:6, 8, and 32) described herein. Such comparisons are generally based on equal concentrations of the molecules being compared. The comparison can also be based on the amount of protein or polypeptide required to reach 50% of the maximal activation obtainable.

In addition to the *don-1* cDNA sequences described above, additional splice variants of the *don-1* gene, and related family members of the *don-1* gene present in the mouse, humans, or other species can be identified and readily isolated without undue experimentation by well known molecular biological techniques given the specific sequences described herein. Further, genes may exist at other genetic loci within the genome that encode proteins which have extensive homology to Don-1 polypeptides or one or more domains of Don-1 polypeptides. These genes can be identified via similar techniques.

For example, to obtain additional splice variants of the *don-1* gene, an oligonucleotide probe based on the cDNA sequences described herein, or fragments thereof, e.g., the nucleotide region encoding the EGF domain can be labeled and used to screen a cDNA library constructed from mRNA obtained from an organism of interest. To obtain additional neuregulin-related genes related to the *don-1* gene, an oligonucleotide probe based on the nucleotide region encoding the TM domain of Don-1, can be used to screen a suitable cDNA library.

- 25 -

The preferred method of labeling is to use  $^{32}\text{P}$ -labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide probe. However other methods may be used to label the  
5 oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Hybridization is performed under stringent conditions. Alternatively, a labeled fragment can be used to screen a genomic library derived from the  
10 organism of interest, again, using appropriately stringent conditions. Such stringent conditions are well known, and will vary predictably depending on the specific organisms from which the library and the labeled sequences are derived.

15 Nucleic acid duplex or hybrid stability is expressed as the melting temperature or  $T_m$ , which is the temperature at which a probe dissociates from a target DNA. This melting temperature is used to define the required stringency conditions. If sequences are to be  
20 identified that are related and substantially identical to the probe, rather than identical, then it is useful to first establish the lowest temperature at which only homologous hybridization occurs with a particular SSC or SSPE concentration. Then assume that 1% mismatching  
25 results in  $1^\circ\text{C}$  decrease in the  $T_m$  and reduce the temperature of the final wash accordingly (for example, if sequences with  $\geq 95\%$  identity with the probe are sought, decrease the final wash temperature by  $5^\circ\text{C}$ ). Note that this assumption is very approximate, and the  
30 actual change in  $T_m$  can be between  $0.5^\circ$  and  $1.5^\circ\text{C}$  per 1% mismatch.

As used herein, high stringency conditions include hybridizing at  $68^\circ\text{C}$  in 5x SSC/5x Denhardt solution/1.0% SDS, or in 0.5 M  $\text{NaHPO}_4$  (pH 7.2)/1 mM EDTA/7% SDS, or in  
35 50% formamide/0.25 M  $\text{NaHPO}_4$  (pH 7.2)/0.25 M NaCl/1 mM

- 26 -

EDTA/7% SDS; and washing in 0.2x SSC/0.1% SDS at room temperature or at 42°C, or in 0.1x SSC/0.1% SDS at 68°C, or in 40 mM NaHPO<sub>4</sub> (pH 7.2)/1 mM EDTA/5% SDS at 50°C, or in 40 mM NaHPO<sub>4</sub> (pH 7.2) 1 mM EDTA/1% SDS at 50°C.

- 5 Moderately stringent conditions include washing in 3x SSC at 42°C. The parameters of salt concentration and temperature can be varied to achieve the desired level of identity between the probe and the target nucleic acid.

For guidance regarding such conditions see, for  
10 example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Springs Harbor Press, N.Y.; and Ausubel et al. (eds.), 1995, Current Protocols in Molecular Biology, (John Wiley & Sons, N.Y.) at Unit 2.10.

- 15 In one approach, appropriate human cDNA libraries can be screened. Such cDNA libraries can, for example, include human breast, human prostate, or fetal human brain or lung cDNA libraries. For example, panels of human breast cells can be screened for *don-1* expression  
20 by, for example, Northern blot analysis. Upon detection of *don-1* transcript, cDNA libraries can be constructed from RNA isolated from the appropriate cell line, utilizing standard techniques well known to those of skill in the art. The human cDNA library can then be  
25 screened with a *don-1* probe to isolate a human *don-1* cDNA. As described below, this method was used to determine the human *don-1* cDNAs in Figs. 2, 4, and 7.

- Alternatively, a human total genomic DNA library can be screened using *don-1* probes. *Don-1*-positive  
30 clones can then be sequenced and, further, the intron/exon structure of the human *don-1* gene can be elucidated. Once genomic sequence is obtained, oligonucleotide primers can be designed based on the sequence for use in the isolation, via, for example  
35 Reverse Transcriptase-coupled PCR, of human *don-1* cDNA.



- 27 -

Further, a previously unknown gene sequence can be isolated by performing PCR using two degenerate oligonucleotide primer pools designed on the basis of nucleotide sequences within the *don-1* cDNAs defined  
5 herein. The template for the reaction can be cDNA obtained by reverse transcription of mRNA prepared from human or non-human cell lines or tissue known or suspected to express a *don-1* gene allele. The PCR product can be subcloned and sequenced to insure that the  
10 amplified sequences represent the sequences of a *don-1* or *don-1*-like gene nucleic acid sequence.

The PCR fragment can then be used to isolate a full length cDNA clone by a variety of methods. For example, the amplified fragment can be labeled and used  
15 to screen a bacteriophage cDNA library. Alternatively, the labeled fragment can be used to screen a genomic library.

PCR technology also can be used to isolate full length cDNA sequences. For example, RNA can be isolated,  
20 following standard procedures, from an appropriate cellular or tissue source. A reverse transcription reaction can be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming of first strand  
25 synthesis. The resulting RNA/DNA hybrid can then be "tailed" with guanines using a standard terminal transferase reaction, the hybrid can be digested with RNAase H, and second strand synthesis can then be primed with a poly-C primer. Thus, cDNA sequences upstream of  
30 the amplified fragment can easily be isolated. For a review of useful cloning strategies, see e.g., Sambrook et al., supra; and Ausubel et al., supra.

In cases where the gene identified is the normal (wild type) gene, this gene can be used to isolate mutant  
35 alleles of the gene. Such an isolation is preferable in

- 28 -

processes and disorders which are known or suspected to have a genetic basis. Mutant alleles can be isolated from individuals either known or suspected to have a genotype which contributes to tumor, e.g.,  
5 adenocarcinoma, proliferation or progression. Mutant alleles and mutant allele gene products can then be utilized in the therapeutic and diagnostic assay systems described below.

A cDNA of a mutant gene can be isolated, for  
10 example, by using PCR, a technique which is well-known to one skilled in the art. In this case, the first cDNA strand can be synthesized by hybridizing a oligo-dT oligonucleotide to mRNA isolated from tissue known or suspected of being expressed in an individual putatively  
15 carrying the mutant allele, and by extending the new strand with reverse transcriptase. The second strand of the cDNA can then be synthesized using an oligonucleotide that hybridizes specifically to the 5'- end of the normal gene. Using these two primers, the product is then  
20 amplified via PCR, cloned into a suitable vector, and subjected to DNA sequence analysis by methods well known in the art. By comparing the DNA sequence of the mutant gene to that of the normal gene, the mutation(s) responsible for the loss or alteration of function of the  
25 mutant gene product can be ascertained.

Alternatively, a genomic or cDNA library can be constructed and screened using DNA or RNA, respectively, from a tissue known to or suspected of expressing the gene of interest in an individual suspected of or known  
30 to carry the mutant allele. The normal gene or any suitable fragment thereof can then be labeled and used as a probe to identify the corresponding mutant allele in the library. The clone containing this gene can then be purified through methods routinely practiced in the art,

- 29 -

and subjected to sequence analysis using standard techniques as described herein.

Additionally, an expression library can be constructed using DNA isolated from or cDNA synthesized  
5 from a tissue known to or suspected of expressing the gene of interest in an individual suspected of or known to carry the mutant allele. In this manner, gene products made by the putatively mutant tissue can be expressed and screened using standard antibody screening  
10 techniques in conjunction with antibodies raised against the normal gene product, as described herein. For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, "Antibodies: A Laboratory Manual," Cold Spring Harbor Press, Cold Spring Harbor.

15 In cases where the mutation results in an expressed gene product with altered function (e.g., as a result of a missense mutation), a polyclonal set of antibodies is likely to cross-react with the mutant gene product. Library clones detected via their reaction with  
20 such labeled antibodies can be purified and subjected to sequence analysis as described herein.

Polypeptides corresponding to one or more domains of full-length Don-1 protein, e.g., the Ig, TM, and EGF domains, are also within the scope of the invention.  
25 Preferred polypeptides are those which are soluble under normal physiological conditions. Also within the invention are fusion proteins in which a portion (e.g., one or more domains) of Don-1 is fused to an unrelated protein or polypeptide (i.e., a fusion partner) to create  
30 a fusion protein. The fusion partner can be a moiety selected to facilitate purification, detection, or solubilization, or to provide some other function. Fusion proteins are generally produced by expressing a hybrid gene in which a nucleotide sequence encoding all  
35 or a portion of Don-1 is joined in-frame to a nucleotide

- 30 -

sequence encoding the fusion partner. Fusion partners include, but are not limited to, the constant region of an immunoglobulin (IgFc). A fusion protein in which a Don-1 polypeptide is fused to IgFc can be more stable and  
5 have a longer half-life in the body than the Don-1 polypeptide on its own.

Also within the scope of the invention are various soluble forms of Don-1. For example, the entire extracellular domain of Don-1 or a portion or domain  
10 thereof can be expressed on its own or fused to a solubilization partner, e.g., an immunoglobulin.

The invention also features Don-1 polypeptides which can inhibit proliferation of adenocarcinoma cells. The ability of the Don-1 polypeptides to inhibit  
15 proliferation of carcinoma cells can be determined using a standard proliferation assay, as follows. Cell, e.g., adenocarcinoma cell, proliferation and viability can be measured by the cleavage of MTT as described by the manufacturer (Boehringer Mannheim, Catalog No. 1465007).  
20 Briefly, cells ( $2 \times 10^3$ ) are seeded in separate 100  $\mu$ L volumes into 96 well tissue culture plates with media containing various concentrations of a Don-1 polypeptide. The plates are then incubated for various times (1 to 3 days) in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. 0.5  
25 mg/ml MTT labeling reagent is added to each well, and the plates are incubated for an additional four hours at 37°C. 100  $\mu$ L of solubilization buffer is then added to each well and the plates are allowed to stand for 12 hours at 37°C. The spectrophotometrical absorbance at  
30 550 and 690 nm is then measured as a gauge of cell proliferation and viability.

In general, Don-1 proteins according to the invention can be produced by transformation  
(transfection, transduction, or infection) of a host cell  
35 with all or part of a Don-1-encoding DNA fragment (e.g.,

- 31 -

one of the cDNAs described herein) in a suitable expression vehicle. Suitable expression vehicles include: plasmids, viral particles, and phage. For insect cells, baculovirus expression vectors are suitable. The entire expression vehicle, or a part thereof, can be integrated into the host cell genome. In some circumstances, it is desirable to employ an inducible expression vector, e.g., the LACSWITCH™ Inducible Expression System (Stratagene; LaJolla, CA).

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems can be used to provide the recombinant protein. The precise host cell used is not critical to the invention. The Don-1 protein can be produced in a prokaryotic host (e.g., *E. coli* or *B. subtilis*) or in a eukaryotic host (e.g., *Saccharomyces* or *Pichia*; mammalian cells, e.g., COS, NIH 3T3 CHO, BHK, 293, or HeLa cells; or insect cells).

Proteins and polypeptides can also be produced in plant cells. For plant cells viral expression vectors (e.g., cauliflower mosaic virus and tobacco mosaic virus) and plasmid expression vectors (e.g., Ti plasmid) are suitable. Such cells are available from a wide range of sources (e.g., the American Type Culture Collection, Rockland, MD; also, see, e.g., Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, 1994). The methods of transformation or transfection and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel et al., supra; expression vehicles may be chosen from those provided, e.g., in *Cloning Vectors: A Laboratory Manual* (P.H. Pouwels et al., 1985, Supp. 1987).

The host cells harboring the expression vehicle can be cultured in conventional nutrient media adapted as

- 32 -

need for activation of a chosen gene, repression of a chosen gene, selection of transformants, or amplification of a chosen gene.

One preferred expression system is the mouse 3T3  
5 fibroblast host cell transfected with a pMAMneo  
expression vector (Clontech, Palo Alto, CA). pMAMneo  
provides an RSV-LTR enhancer linked to a dexamethasone-  
inducible MMTV-LTR promotor, an SV40 origin of  
10 replication which allows replication in mammalian  
systems, a selectable neomycin gene, and SV40 splicing  
and polyadenylation sites. DNA encoding a Don-1 protein  
would be inserted into the pMAMneo vector in an  
orientation designed to allow expression. The  
recombinant Don-1 protein would be isolated as described  
15 below. Other preferable host cells that can be used in  
conjunction with the pMAMneo expression vehicle include  
COS cells and CHO cells (ATCC Accession Nos. CRL 1650 and  
CCL 61, respectively).

Don-1 polypeptides can be produced as fusion  
20 proteins. For example, the expression vector pUR278  
(Ruther et al., *EMBO J.* 2:1791, 1983), can be used to  
create lacZ fusion proteins. The pGEX vectors can be  
used to express foreign polypeptides as fusion proteins  
with glutathione S-transferase (GST). In general, such  
25 fusion proteins are soluble and can be easily purified  
from lysed cells by adsorption to glutathione-agarose  
beads followed by elution in the presence of free  
glutathione. The pGEX vectors are designed to include  
thrombin or factor Xa protease cleavage sites so that the  
30 cloned target gene product can be released from the GST  
moiety.

In an insect cell expression system, Autographa  
californica nuclear polyhydrosis virus (AcNPV), which  
grows in Spodoptera frugiperda cells, is used as a vector  
35 to express foreign genes. A Don-1 coding sequence can be

- 33 -

cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter, e.g., the polyhedrin promoter. Successful insertion of a gene encoding a Don-  
5 1 polypeptide or protein will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat encoded by the polyhedrin gene). These recombinant viruses are then used to infect *spodoptera frugiperda*  
10 cells in which the inserted gene is expressed (see, e.g., Smith et al., *J. Virol.* 46:584, 1983; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems can be utilized. When an adenovirus  
15 is used as an expression vector, the Don-1 nucleic acid sequence can be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene can then be inserted into the adenovirus genome by *in vitro* or *in*  
20 *vivo* recombination. Insertion into a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a Don-1 gene product in infected hosts (see, e.g., Logan, *Proc. Natl. Acad. Sci. USA* 81:3655,  
25 1984).

Specific initiation signals may be required for efficient translation of inserted nucleic acid sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire native Don-  
30 1 gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. In other cases, exogenous translational control signals, including, perhaps, the  
35 ATG initiation codon, must be provided. Furthermore, the

- 34 -

initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of  
5 origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators (Bittner et al., Methods in Enzymol. 153:516, 1987).

10 In general, the signal sequence can be a component of the expression vector, or it may be a part of *don-1* DNA that is inserted into the vector. The native *don-1* DNA is thought to encode a signal sequence at the amino terminus of the polypeptide that is cleaved during post-  
15 translational processing to form the mature Don-1 polypeptide that binds to the p185 receptor. However, a conventional signal structure is not apparent. Native Don-1 is secreted from cells, but may remain lodged in the membrane because it contains a transmembrane domain  
20 and a cytoplasmic region in the carboxyl terminal region of the polypeptide. Thus, in a secreted, soluble version of Don-1, the carboxyl terminal domain of the molecule, including the transmembrane domain, is ordinarily deleted. This truncated form of the Don-1 polypeptide  
25 may be secreted from the cell, provided that the DNA encoding the truncated variant encodes a signal sequence recognized by the host.

Don-1 polypeptides can be expressed directly or as a fusion with a heterologous polypeptide, such as a  
30 signal sequence or other polypeptide having a specific cleavage site at the N-and/or C-terminus of the mature protein or polypeptide. Included within the scope of this invention are Don-1 polypeptides with the native signal sequence deleted and replaced with a heterologous  
35 signal sequence. The heterologous signal sequence



- 35 -

selected should be one that is recognized and processed, i.e., cleaved by a signal peptidase, by the host cell. For prokaryotic host cells that do not recognize and process the native Don-1 signal sequence, the signal  
5 sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native Don-1 signal sequence may be substituted by the yeast  
10 invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

A host cell may be chosen which modulates the  
15 expression of the inserted sequences, or modifies and processes the gene product in a specific, desired fashion. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different  
20 host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein  
25 expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such mammalian host cells include, but are not limited to,  
30 CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, choroid plexus cell lines.

Alternatively, a Don-1 protein can be produced by a stably-transfected mammalian cell line. A number of vectors suitable for stable transfection of mammalian  
35 cells are available to the public, see, e.g., Pouwels et

- 36 -

al. (supra); methods for constructing such cell lines are also publicly available, e.g., in Ausubel et al. (supra). In one example, cDNA encoding the Don-1 protein is cloned into an expression vector that includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, the Don-1 protein-encoding gene into the host cell chromosome is selected for by including 0.01-300  $\mu$ M methotrexate in the cell culture medium (as described in Ausubel et al., supra). This dominant selection can be accomplished in most cell types.

Recombinant protein expression can be increased by DHFR-mediated amplification of the transfected gene. Methods for selecting cell lines bearing gene amplifications are described in Ausubel et al. (supra); such methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. DHFR-containing expression vectors commonly used for this purpose include pCVSEII-DHFR and pAdD26SV(A) (described in Ausubel et al., supra). Any of the host cells described above or, preferably, a DHFR-deficient CHO cell line (e.g., CHO DHFR<sup>-</sup> cells, ATCC Accession No. CRL 9096) are among the host cells preferred for DHFR selection of a stably-transfected cell line or DHFR-mediated gene amplification.

A number of other selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase, and adenine phosphoribosyltransferase genes can be employed in tk, hgp<sup>r</sup>t, or ap<sup>r</sup>t cells, respectively. In addition, g<sup>p</sup>t, which confers resistance to mycophenolic acid (Mulligan et al., *Proc. Natl. Acad. Sci. USA*, 78:2072, 1981); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin et al., *J. Mol. Biol.*, 150:1, 1981); and hyg<sup>r</sup>o, which confers

- 37 -

resistance to hygromycin (Santerre et al., *Gene*, **30**:147, 1981), can be used.

Alternatively, any fusion protein can be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described in Janknecht et al., *Proc. Natl. Acad. Sci. USA*, **88**:8972 (1981), allows for the ready purification of non-denatured fusion proteins expressed in human cell lines. In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni<sup>2+</sup> nitriloacetic acid-agarose columns, and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

Alternatively, Don-1 or a portion thereof, can be fused to an immunoglobulin Fc domain. Such a fusion protein can be readily purified using a protein A column. Moreover, such fusion proteins permit the production of a dimeric form of a Don-1 polypeptide having increased stability *in vivo*.

Don-1 proteins and polypeptides can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, and non-human primates, e.g., baboons, monkeys, and chimpanzees, can be used to generate Don-1-expressing transgenic animals. Various known techniques can be used to introduce a *don-1* transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten et al., *Proc. Natl. Acad. Sci., USA*, **82**:6148, 1985); gene targeting into embryonic stem

- 38 -

cells (Thompson et al., *Cell*, **56**:313, 1989); and electroporation of embryos (Lo, *Mol. Cell. Biol.*, **3**:1803, 1983).

The present invention provides for transgenic  
5 animals that carry the *don-1* transgene in all their cells, as well as animals that carry the transgene in some, but not all of their cells, i.e., mosaic animals. The transgene can be integrated as a single transgene or in concatamers, e.g., head-to-head tandems or head-to-  
10 tail tandems. The transgene can also be selectively introduced into and activated in a particular cell type (Lasko et al., *Proc. Natl. Acad. Sci. USA*, **89**:6232, 1992). The regulatory sequences required for such a cell-type specific activation will depend upon the  
15 particular cell type of interest, and will be apparent to those of skill in the art.

When it is desired that the *don-1* transgene be integrated into the chromosomal site of the endogenous *don-1* gene, gene targeting is preferred. Briefly, when  
20 such a technique is to be used, vectors containing some nucleotide sequences homologous to an endogenous *don-1* gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of  
25 the endogenous gene. The transgene also can be selectively introduced into a particular cell type, thus inactivating the endogenous *don-1* gene in only that cell type (Gu et al., *Science*, **265**:103, 1984). The regulatory sequences required for such a cell-type specific  
30 inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant *don-1* gene can be assayed  
35 utilizing standard techniques. Initial screening may be

- 39 -

accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to assay whether integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the  
5 transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and RT-PCR. Samples of *don-1* gene-expressing tissue, also can be evaluated  
10 immunocytochemically using antibodies specific for the Don-1 transgene product.

Once the recombinant Don-1 protein is expressed, it is isolated. Secreted forms can be isolated from the culture media, while non-secreted forms must be isolated  
15 from the host cells. Proteins can be isolated by affinity chromatography. In one example, an anti-Don-1 protein antibody (e.g., produced as described herein) is attached to a column and used to isolate the Don-1 protein. Lysis and fractionation of Don-1 protein-  
20 harboring cells prior to affinity chromatography can be performed by standard methods (see, e.g., Ausubel et al., supra). Alternatively, a Don-1 fusion protein, for example, a Don-1-maltose binding protein, a Don-1- $\beta$ -galactosidase, or a Don-1-trpE fusion protein, can be  
25 constructed and used for Don-1 protein isolation (see, e.g., Ausubel et al., supra; New England Biolabs, Beverly, MA).

Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance  
30 liquid chromatography using standard techniques (see, e.g., Fisher, *Laboratory Techniques In Biochemistry And Molecular Biology*, eds., Work and Burdon, Elsevier, 1980).

Given the amino acid sequences described herein,  
35 polypeptides of the invention, particularly short Don-1

- 40 -

polypeptides, can be produced by standard chemical synthesis (e.g., by the methods described in *Solid Phase Peptide Synthesis*, 2nd ed., The Pierce Chemical Co., Rockford, IL, 1984).

5           These general techniques of polypeptide expression and purification can also be used to produce and isolate useful Don-1 polypeptide analogs (described herein).

          The invention also features proteins which interact with Don-1 and are involved in the function of  
10 Don-1. Also included in the invention are the genes encoding these interacting proteins. Interacting proteins can be identified using methods known to those skilled in the art. One suitable method is the "two-hybrid system," which detects protein interactions in  
15 vivo (Chien et al., *Proc. Natl. Acad. Sci. USA*, **88**:9578, 1991). A kit for practicing this method is available from Clontech (Palo Alto, CA).

#### Anti-Don-1 Antibodies

          Human Don-1 proteins and polypeptides (or  
20 immunogenic fragments or analogs) can be used to raise antibodies useful in the invention, and such polypeptides can be produced by recombinant or peptide synthetic techniques (see, e.g., *Solid Phase Peptide Synthesis*, supra; Ausubel et al., supra). In general, the peptides  
25 can be coupled to a carrier protein, such as KLH, as described in Ausubel et al., supra, mixed with an adjuvant, and injected into a host mammal. Antibodies can be purified by peptide antigen affinity chromatography.

30           In particular, various host animals can be immunized by injection with a Don-1 protein or polypeptide. Host animals include rabbits, mice, guinea pigs, and rats. Various adjuvants can be used to increase the immunological response, depending on the

- 41 -

host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of the immunized animals.

10       Antibodies within the invention include monoclonal antibodies, polyclonal antibodies, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')<sub>2</sub> fragments, and molecules produced using a Fab expression library.

15       Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, can be prepared using the Don-1 proteins described above and standard hybridoma technology (see, e.g., Kohler et al., *Nature*, 256:495, 1975; Kohler et al., *Eur. J. Immunol.*, 6:511, 1976; Kohler et al., *Eur. J. Immunol.*, 6:292, 1976; Hammerling et al., In Monoclonal Antibodies and T Cell Hybridomas, Elsevier, NY, 1981; Ausubel et al., supra).

25       In particular, monoclonal antibodies can be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture such as described in Kohler et al., *Nature*, 256:495, 1975, and U.S. Patent No. 4,376,110; the human B-cell hybridoma technique (Kosbor et al., *Immunology Today*, 4:72, 1983; Cole et al., *Proc. Natl. Acad. Sci. USA*, 80:2026, 1983), and the EBV-hybridoma technique (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96, 1983). Such antibodies can be of any immunoglobulin class including IgG, IgM, 35 IgE, IgA, IgD, and any subclass thereof. The hybridoma

- 42 -

producing the mAb of this invention can be cultivated *in vitro* or *in vivo*. The ability to produce high titers of mAbs *in vivo* makes this the presently preferred method of production.

5           Once produced, polyclonal or monoclonal antibodies are tested for specific Don-1 recognition by Western blot or immunoprecipitation analysis by standard methods, e.g., as described in Ausubel et al., supra. Antibodies that specifically recognize and bind to Don-1 are useful  
10 in the invention. For example, such antibodies can be used in an immunoassay to monitor the level of Don-1 produced by a mammal (for example, to determine the amount or subcellular location of Don-1).

          Preferably, antibodies of the invention are  
15 produced using fragments of the Don-1 protein which lie outside highly conserved regions and appear likely to be antigenic, by criteria such as high frequency of charged residues. In one specific example, such fragments are generated by standard techniques of PCR, and are then  
20 cloned into the pGEX expression vector (Ausubel et al., supra). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel, et al., supra.

          Antibodies can also be prepared to bind  
25 specifically to one or more particular domains of Don-1, such as the EGF domain (SEQ ID NO:11), by immunizing an animal with a polypeptide corresponding to only the desired domain or domains.

          In some cases it may be desirable to minimize the  
30 potential problems of low affinity or specificity of antisera. In such circumstances, two or three fusions can be generated for each protein, and each fusion can be injected into at least two rabbits. Antisera can be raised by injections in a series, preferably including at  
35 least three booster injections.



- 43 -

Antisera is also checked for its ability to immunoprecipitate recombinant Don-1 proteins or control proteins, such as glucocorticoid receptor, CAT, or luciferase.

- 5           The antibodies can be used, for example, in the detection of the Don-1 in a biological sample as part of a diagnostic assay. Antibodies also can be used in a screening assay to measure the effect of a candidate compound on expression or localization of Don-1.
- 10       Additionally, such antibodies can be used in conjunction with the gene therapy techniques described to, for example, evaluate the normal and/or engineered Don-1-expressing cells prior to their introduction into the patient. Such antibodies additionally can be used in a
- 15       method for inhibiting abnormal Don-1 activity.

Techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci.*, **81**:6851, 1984; Neuberger et al., *Nature*, **312**:604, 1984; Takeda et al., *Nature*, **314**:452, 1984) can be used

20       to splice the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity. A chimeric antibody is a molecule in which different portions are derived from different animal

25       species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent

30       4,946,778; and U.S. Patents 4,946,778 and 4,704,692) can be adapted to produce single chain antibodies against a Don-1 protein or polypeptide. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a

35       single chain polypeptide.

- 44 -

Antibody fragments that recognize and bind to specific epitopes can be generated by known techniques. For example, such fragments can include but are not limited to  $F(ab')_2$  fragments, which can be produced by  
5 pepsin digestion of the antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of  $F(ab')_2$  fragments. Alternatively, Fab expression libraries can be constructed (Huse et al., *Science*, **246**:1275, 1989) to allow rapid and easy  
10 identification of monoclonal Fab fragments with the desired specificity.

Antibodies to Don-1 can, in turn, be used to generate anti-idiotypic antibodies that resemble a portion of Don-1, using techniques well known to those skilled in  
15 the art (see, e.g., Greenspan et al., *FASEB J.*, **7**:437, 1993; Nissinoff, *J. Immunol.*, **147**:2429, 1991). For example, antibodies that bind to Don-1 and competitively inhibit the binding of a ligand of Don-1 can be used to generate anti-idiotypes that resemble a ligand binding  
20 domain of Don-1 and, therefore, bind and neutralize a ligand of Don-1. Such neutralizing anti-idiotypic antibodies or Fab fragments of such anti-idiotypic antibodies can be used in therapeutic regimens.

In addition, antibodies can be expressed within an  
25 intracellular compartment of a cell, such as the endoplasmic reticulum, to specifically bind to a target protein or polypeptide within the cell. Such specific binding can be used to alter, e.g., inhibit, the function of the target protein. Intracellular expression of  
30 antibodies is achieved by introducing into the cells nucleic acids that encode the antibodies, e.g., by using a recombinant viral vector or other vector system suitable for delivering a gene to a cell *in vivo*.

Preferably the antibody is a single chain Fv  
35 fragment, although whole antibodies, or antigen binding

- 45 -

fragments thereof, e.g., Fab fragments, can be used. Targeting of an antibody to an intracellular compartment can be accomplished by incorporating an appropriate signal sequence into the antibody. For example, a  
5 nucleic acid can be designed to include a first nucleotide sequence encoding a signal sequence (e.g., to an endoplasmic reticulum), operatively linked in a 5' to 3' direction by a phosphodiester bond to a second  
10 nucleotide sequence encoding a single chain Fv fragment that binds to a Don-1 polypeptide. These techniques are described in detail in Curiel et al., PCT Publication No. WO 96/07321.

#### Modulating Don-1 Expression

Don-1 polypeptides can be administered to  
15 stimulate the proliferation of cells, such as epithelial cells, e.g., to promote wound healing. Other therapies, e.g., anti-tumor therapies, can be designed to reduce the level of endogenous Don-1 gene expression, e.g., using antisense or ribozyme approaches to inhibit or prevent  
20 translation of Don-1 mRNA transcripts; triple helix approaches to inhibit transcription of the Don-1 gene; or targeted homologous recombination to inactivate or "knock out" the Don-1 gene or its endogenous promoter.

Because the Don-1 gene is expressed in the brain,  
25 delivery techniques should be preferably designed to cross the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134). Alternatively, the antisense, ribozyme, or DNA constructs described herein could be administered directly to the site containing the target cells; e.g.,  
30 brain, kidney, lung, uterus, endothelial and epithelial cells, fibroblasts, and breast and prostate cells.

#### Antisense Nucleic Acids

Antisense approaches involve the design of oligonucleotides (either DNA or RNA) that are

- 46 -

complementary to Don-1 mRNA. The antisense oligonucleotides bind to the complementary Don-1 mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. A  
5 sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA and form a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex  
10 DNA can be tested, or triplex formation can be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may  
15 contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

20 Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences  
25 of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well (Wagner, *Nature*, 372:333, 1984). Thus, oligonucleotides complementary to either the 5'- or 3'- non-translated, non-coding regions of the *don-1* gene, e.g., the human gene, as represented by the  
30 cDNA (SEQ ID NO:5) shown in Fig. 3, can be used in an antisense approach to inhibit translation of endogenous Don-1 mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon.

- 47 -

Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'-, or coding region of Don-1 mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression.

It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA, or chimeric mixtures, or derivatives or modified versions thereof, and can be single-stranded or double-stranded. The oligonucleotides can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to

- 48 -

improve stability of the molecule, hybridization, etc.  
The oligonucleotide may include other appended groups  
such as peptides (e.g., for targeting host cell receptors  
*in vivo*), or agents facilitating transport across the  
5 cell membrane (as described, e.g., in Letsinger et al.,  
*Proc. Natl. Acad. Sci. USA*, **86**:6553, 1989; Lemaitre et  
al., *Proc. Natl. Acad. Sci. USA*, **84**:648, 1987; PCT  
Publication No. WO 88/09810) or the blood-brain barrier  
(see, e.g., PCT Publication No. WO 89/10134), or  
10 hybridization-triggered cleavage agents (see, e.g., Krol  
et al., *BioTechniques*, **6**:958, 1988), or intercalating  
agents (see, e.g., Zon, *Pharm. Res.*, **5**:539, 1988). To  
this end, the oligonucleotide can be conjugated to  
another molecule, e.g., a peptide, hybridization  
15 triggered cross-linking agent, transport agent, or  
hybridization-triggered cleavage agent.

The antisense oligonucleotide can include at least  
one modified base moiety selected from the group  
including, but not limited to, 5-fluorouracil, 5-  
20 bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine,  
xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl)  
uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-  
carboxymethyl-aminomethyluracil, dihydrouracil, beta-D-  
galactosylqueosine, inosine, N6-isopentenyladenine, 1-  
25 methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-  
methyladenine, 2-methylguanine, 3-methylcytosine, 5-  
methylcytosine, N6-adenine, 7-methylguanine, 5-  
methylaminomethyluracil, 5-methoxyaminomethyl-2-  
thiouracil, beta-D-mannosylqueosine, 5'-  
30 methoxycarboxymethyluracil, 5-methoxyuracil, 2-  
methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid  
(v), wybutoxosine, pseudouracil, queosine, 2-  
thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-  
thiouracil, 5-methyluracil, uracil-5-oxyacetic acid  
35 methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-

- 49 -

thiouracil, 2-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide can also include at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide includes at least one modified phosphate backbone, e.g., a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal, or an analog of any of these backbones.

In addition, the antisense oligonucleotide can be an  $\alpha$ -anomeric oligonucleotide that forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gautier et al., *Nucl. Acids. Res.*, 15:6625, 1987). The oligonucleotide can be a 2'-O-methylribonucleotide (Inoue et al., *Nucl. Acids Res.*, 15:6131, 1987), or a chimeric RNA-DNA analog (Inoue et al., *FEBS Lett.*, 215:327, 1987).

Antisense oligonucleotides of the invention can be synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein et al., *Nucl. Acids Res.*, 16:3209, 1988, and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., *Proc. Natl. Acad. Sci. USA*, 85:7448, 1988).

While antisense nucleotides complementary to the Don-1 coding region sequence could be used, those

- 50 -

complementary to the transcribed untranslated region are most preferred.

One example of a 15 nucleotide antisense sequence to the human *don-1* gene is directed against the EGF domain: 5'-GACTTGGCTCTCTCG-3' (SEQ ID NO:22). Another example of a 15 nucleotide antisense sequence to the human *don-1* gene is: 5'-GGACTCCGACATTCT-3' (SEQ ID NO:23), where the underlined sequence represents the complement of the initiator methionine codon.

The antisense molecules should be delivered to cells that express Don-1 *in vivo*, e.g., brain, kidney, lung, uterus, endothelial and epithelial cells, fibroblasts, and breast and prostate cells. A number of methods have been developed for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense molecules sufficient to suppress translation of endogenous mRNAs. Therefore, a preferred approach uses a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous Don-1 transcripts and thereby prevent translation of the Don-1 mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally



- 51 -

integrated, as long as it can be transcribed to produce the desired antisense RNA.

Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be  
5 plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible  
10 or constitutive. Such promoters include, but are not limited to: the SV40 early promoter region (Berndt et al., *Nature*, 290:304, 1981); the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell*, 22:787-797, 1988); the herpes  
15 thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. USA*, 78:1441, 1981); or the regulatory sequences of the metallothionein gene (Brinster et al., *Nature*, 296:39, 1988).

Any type of plasmid, cosmid, YAC, or viral vector  
20 can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site; e.g., the brain, kidney, lung, uterus, endothelial and epithelial cells, fibroblasts, and breast and prostate cells. Alternatively, viral vectors can be used that  
25 selectively infect the desired tissue (e.g., for brain, herpesvirus vectors may be used), in which case administration can be accomplished by another route (e.g., systemically).

#### Ribozymes

30 Ribozyme molecules designed to catalytically cleave Don-1 mRNA transcripts also can be used to prevent translation of Don-1 mRNA and expression of Don-1 (see, e.g., PCT Publication WO 90/11364; Saraver et al., *Science*, 247:1222, 1990). While various ribozymes that  
35 cleave mRNA at site-specific recognition sequences can be

- 52 -

used to destroy Don-1 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole  
5 requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is known in the art (Haseloff et al., *Nature*, 334:585, 1988). There are numerous examples of potential hammerhead ribozyme  
10 cleavage sites within the nucleotide sequence of human Don-1 cDNAs (Figs. 2 and 4). Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the Don-1 mRNA, i.e., to increase efficiency and minimize the intracellular  
15 accumulation of non-functional mRNA transcripts.

Examples of potential ribozyme sites in human Don-1 include 5'-UG-3' sites which correspond to the initiator methionine codon (nucleotides 664-666 in human SEQ ID NO:5 and 69-71 in human SEQ ID NOs:7 and 31) and  
20 the codons for each of the cysteine residues of the EGF domain (e.g., nucleotides 493-494, 517-519, 535-537, 568-570, 574-576, and 601-603 in human SEQ ID NOs:7 and 31, and nucleotides 973-975, 997-999, 1015-1017, 1048-1050, 1054-1056, and 1081-1083 in human SEQ ID NO:5).

25 The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes"), such as the one that occurs naturally in *Tetrahymena Thermophila* (known as the IVS or L-19 IVS RNA), and which has been extensively described by Cech  
30 and his collaborators (Zaug et al., *Science*, 224:574, 1984; Zaug et al., *Science*, 231:470, 1986; Zug et al., *Nature*, 324:429, 1986; PCT Application No. WO 88/04300; and Been et al., *Cell*, 47:207, 1986). The Cech-type ribozymes have an eight base-pair sequence that  
35 hybridizes to a target RNA sequence, whereafter cleavage

- 53 -

of the target RNA takes place. The invention encompasses those Cech-type ribozymes that target eight base-pair active site sequences present in Don-1 polypeptides.

As in the antisense approach, the ribozymes can be  
5 composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.), and should be delivered to cells which express the Don-1 *in vivo*, e.g., brain, kidney, lung, uterus, endothelial and epithelial cells, fibroblasts, and breast and prostate cells. A preferred  
10 method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive *pol* III or *pol* II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous Don-1 messages and  
15 inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

#### Other Methods for Reducing Don-1 Expression

Endogenous *don-1* gene expression can also be  
20 reduced by inactivating or "knocking out" the *don-1* gene or its promoter using targeted homologous recombination (see, e.g., U.S. Patent No. 5,464,764). For example, a mutant, non-functional *don-1* (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous  
25 *don-1* gene (either the coding regions or regulatory regions of the *don-1* gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express Don-1 *in vivo*. Insertion of the DNA construct, via targeted homologous recombination,  
30 results in inactivation of the *don-1* gene. Such approaches are particularly suited for use in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive *don-1* gene. This approach can be adapted for  
35 use in humans, provided the recombinant DNA constructs

- 54 -

are directly administered or targeted to the required site *in vivo* using appropriate viral vectors, e.g., herpes virus vectors for delivery to brain tissue.

Alternatively, endogenous *don-1* gene expression  
5 can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the *don-1* gene (i.e., *don-1* promoters and/or enhancers located upstream to the start codon in the untranslated region) to form  
10 triple helical structures that prevent transcription of the *don-1* gene in target cells in the body (Helene, *Anticancer Drug Des.*, 6:569, 1981; Helene et al., *Ann. N.Y. Acad. Sci.*, 660:27, 1992; and Maher, *Bioassays*, 14:807, 1992).

#### Identification of Proteins That Interact With Don-1

15 The invention also features proteins that interact with Don-1 polypeptides. Any method suitable for detecting protein-protein interactions can be employed to identify transmembrane, intracellular, or extracellular proteins that interact with Don-1 polypeptides. Among  
20 the traditional methods which can be employed are co-immunoprecipitation, crosslinking and co-purification through gradients or chromatographic columns of cell lysates or proteins obtained from cell lysates, and the use of Don-1 polypeptides to identify proteins in the  
25 lysate that interact with the Don-1 polypeptide.

For these assays, the Don-1 polypeptide can be a full length Don-1, a soluble extracellular domain of Don-1, or some other suitable Don-1 polypeptide, e.g., a polypeptide including the EGF domain of Don-1. Once  
30 isolated, such an interacting protein can be identified and cloned and then used, in conjunction with standard techniques, to identify proteins with which it interacts. For example, at least a portion of the amino acid sequence of a protein which interacts with a Don-1

- 55 -

polypeptide can be ascertained using techniques well known to those of skill in the art, such as via the Edman degradation technique. The amino acid sequence obtained can be used as a guide to generate oligonucleotide mixtures that can be used to screen for gene sequences encoding the interacting protein. Screening can be accomplished, for example, by standard hybridization or PCR techniques. Techniques for generating oligonucleotide mixtures and the screening are known.

5  
10 See, e.g., Ausubel, *supra*; and PCR Protocols: A Guide to Methods and Applications, 1990, Innis et al., eds. Academic Press, Inc., New York.

Additionally, methods may be employed which result in the direct identification of genes that encode proteins that interact with Don-1 polypeptides. These methods include, for example, screening expression libraries, in a manner similar to the well known technique of antibody probing of  $\lambda$ gt11 libraries, using a labeled Don-1 polypeptide or a Don-1 fusion protein, e.g., a Don-1 domain fused to a marker such as an enzyme, fluorescent dye, a luminescent protein, or to an IgFc domain.

15  
20

There are also methods for detecting protein interactions, e.g., the *in vivo* two-hybrid system (Chien et al., *Proc. Natl. Acad. Sci. USA*, **88**:9578, 1991). A kit for practicing this method is available from Clontech (Palo Alto, CA). Briefly, to use this system, plasmids are constructed that encode two hybrid proteins. One plasmid includes a nucleotide sequence encoding the DNA-binding domain of a transcription activator protein fused to a nucleotide sequence encoding a full-length Don-1 protein, a Don-1 polypeptide, or a Don-1 fusion protein. The other plasmid includes a nucleotide sequence encoding the transcription activator protein's activation domain fused to a cDNA encoding an unknown protein from which a

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- 56 -

cDNA library has been recombined into this plasmid. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (e.g., HBS or  
5 *lacZ*) whose regulatory region contains the transcription activator's binding site.

Either hybrid protein alone cannot activate transcription of the reporter gene. The DNA-binding domain hybrid cannot because it does not provide  
10 activation function, and the activation domain hybrid cannot because it cannot localize to the activator's binding sites. Interaction of the appropriate two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is  
15 detected by an assay for the reporter gene product.

The two-hybrid system and related methods can be used to screen activation domain libraries for proteins that interact with a "bait" gene product. By way of example, a Don-1 polypeptide can be used as the bait gene  
20 product. Total genomic or cDNA sequences are fused to DNA encoding an activation domain. This library and a plasmid encoding a hybrid of bait Don-1 gene product fused to the DNA-binding domain are cotransformed into a yeast reporter strain, and the resulting transformants  
25 are screened for those that express the reporter gene. For example, a bait *don-1* gene sequence encoding a Don-1 polypeptide, or a domain of Don-1, can be cloned into a vector such that it is translationally fused to DNA encoding the DNA-binding domain of the GAL4 protein.  
30 These colonies are purified and the library plasmids responsible for reporter gene expression are isolated. DNA sequencing is then used to identify the proteins encoded by the library plasmids.

A cDNA library of the cell line from which  
35 proteins that interact with bait *don-1* gene product are

- 57 -

to be detected can be made using methods routinely practiced in the art. According to the particular system described herein, for example, cDNA fragments can be inserted into a vector such that they are translationally  
5 fused to the transcriptional activation domain of GAL4. This library can be co-transformed along with the bait *don-1* gene-GAL4 fusion plasmid into a yeast strain which contains a *lacZ* gene driven by a promoter that contains a GAL4 activation sequence. A cDNA encoded protein, fused  
10 to GAL4 transcriptional activation domain, that interacts with bait *don-1* gene product will reconstitute an active GAL4 protein and thereby drive expression of the *HIS3* gene. Colonies that express *HIS3* then can be purified from these strains, and used to produce and isolate the  
15 bait *don-1* gene-interacting protein using techniques routinely practiced in the art.

#### Therapeutic Applications

The Don-1 proteins and polypeptides described herein stimulate proliferation of epithelial cells and  
20 are thus particularly implicated in melanomas and adenocarcinomas in which epithelial cells proliferate out of control. Accordingly, undesirable tumors, such as melanomas and adenocarcinomas of the skin, esophagus, lung, breast, liver, pancreas, gastrointestinal tract,  
25 colon, prostate, and uterus can be reduced by the administration of a compound that interferes with Don-1 expression or function (e.g., an antibody). Compounds that interfere with Don-1 function can also be used to treat other undesirable disease processes, e.g., cyst and  
30 polyp formation.

In addition, since Don-1 polypeptides promote or stimulate epithelial cell proliferation, the topical administration of Don-1 polypeptides to wounds promotes wound healing.

- 58 -

Because Don-1 is highly expressed in the brain, Don-1 also may play a significant role regulating tumor formation and progression in the brain. Of course, in some circumstances, including certain phases of many of the above-described conditions, it may be desirable to enhance Don-1 function, e.g., to stimulate cell proliferation or differentiation, or enhance or suppress apoptosis.

Recombinant Don-1 should facilitate the production of pharmacologic modifiers and inhibitors of Don-1 function. Compounds that interfere with Don-1 function include molecules that bind to Don-1, such as antibodies, and prevent it from binding with its receptors, e.g., p185, or small molecules or anti-idiotypic antibodies, that mimic certain domains of Don-1, such as the EGF domain, and bind, preferably irreversibly, to Don-1 receptors without activating these receptors, e.g., without causing phosphorylation or dimerization of these receptors. For example, using standard techniques, a Don-1 EGF polypeptide can be mutated and tested in the p185 assay described herein. Any of these mutant polypeptides that bind to the receptor with high affinity, but do not cause phosphorylation and/or dimerization, are candidates for anti-tumor therapy.

Therapeutic Don-1 polypeptides, antibodies, or small molecules of the invention can be administered by any appropriate route, e.g., injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional routes, or by sustained release systems as note below. Don-1 is administered continuously by infusion or by bolus injection. Don-1 antibodies are administered in the same fashion, or by administration into the blood stream or lymph. Treatment is repeated as necessary for alleviation of disease symptoms.



- 59 -

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the protein, which matrices are in the form of shaped articles, e.g., films or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (e.g., poly(2-hydroxyethyl-methacrylate) as described by Langer et al., *J. Biomed. Mater. Res.*, **15**:167-277 (1981), and Langer, *Chem. Tech.*, **12**:98-105 (1982), or polyvinylalcohol), or polylactides (as described in U.S. Pat. No. 3,773,919, and EPA 58,481).

Sustained-release Don-1 polypeptide or antibody compositions also include liposomally entrapped Don-1 or Don-1 antibodies. Liposomes containing Don-1 or antibody are prepared by methods known per se. See, e.g., Epstein et al., *P.N.A.S., USA*, **82**:3688-3692 (1985); Hwang et al., *P.N.A.S., USA*, **77**:4030-4034 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. The liposomes are preferably about 200-800 Angstroms in diameter and are unilamellar. The lipid content is generally greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Don-1 therapy. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556.

An effective amount of Don-1 or Don-1 antibody to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage might range from about 1.0  $\mu\text{g/kg}$  to about 100 mg/kg or more, depending on the factors mentioned above. Typically, the clinician will administer Don-1 or Don-1 antibody until a dosage is

- 60 -

reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays.

#### Diagnostic Applications

5           The polypeptides of the invention and the antibodies specific for these polypeptides are also useful for identifying those compartments of mammalian cells that contain proteins important to the function of Don-1. Antibodies specific for Don-1 can be produced as  
10 described above. The normal subcellular location of the protein is then determined either *in situ* or using fractionated cells by any standard immunological or immunohistochemical procedure (see, e.g., Ausubel et al., supra; Bancroft and Stevens, Theory and Practice of  
15 Histological Techniques, Churchill Livingstone, 1982).

          Antibodies specific for Don-1 also can be used to detect or monitor Don-1-related diseases. For example, levels of a Don-1 protein in a sample can be assayed by any standard technique using these antibodies. For  
20 example, Don-1 protein expression can be monitored by standard immunological or immunohistochemical procedures (e.g., those described above) using the antibodies described herein. Alternatively, Don-1 expression can be assayed by standard Northern blot analysis or can be  
25 aided by PCR (see, e.g., Ausubel et al., supra; PCR Technology: Principles and Applications for DNA Amplification, ed., H.A. Ehrlich, Stockton Press, NY). If desired or necessary, analysis can be carried out to detect point mutations in the Don-1 sequence (for  
30 example, using well known nucleic acid mismatch detection techniques). All of the above techniques are enabled by the Don-1 sequences described herein.

- 61 -

Examples

Example 1 describes the identification and sequencing of several cDNAs corresponding to different splice variants of murine and human *don-1* genes. Example 2 describes the characterization of Don-1 using a p185 assay, and differential expression pattern experiments. Example 3 describes chromosomal mapping of the *don-1* gene.

Example 1: Cloning of the *don-1* Gene

10 The gene for murine Don-1 was identified in a mouse choroid plexus cDNA library. The first murine splice variant of the *don-1* gene was used to identify an additional murine splice variant in a mouse lung cDNA library and two splice variants of the human *don-1* gene  
15 in a human fetal lung cDNA library. The identification and sequencing of both murine and human genes is described in this first example.

cDNA Library Screening

To obtain a full length cDNA sequence, a mouse  
20 lung library (Stratagene, La Jolla, Ca) was screened using the 1.4 kb Not I/Sal I fragment originally isolated from a choroid plexus library as described below. Screening protocols were as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., (Cold  
25 Spring Harbor Press, 1989). A homologous human sequence was obtained from a human fetal brain library (Clontech, Palo Alto, Ca) by hybridization with a 1.4 kb NotI/SalI fragment of the murine cDNA of SEQ ID NO:1 as described above.

30 Choroid-Plexus mRNA Isolation

The murine mRNA used to create the murine choroid plexus library was prepared as follows. Total RNA was isolated from mouse choroid plexus tissue using the guanidinium isothiocyanate/CsCl method of Chirgwin et al.

- 62 -

(*Biochemistry* 18:5294, 1979) as described in Current Protocols for Molecular Biology (*supra*). The RNA was quantitated, diluted to 1 mg/ml in water, and then incubated for 30 minutes at 37°C with an equal volume of  
5 DNase solution (20 mM MgCl<sub>2</sub>, 2 mM DTT, 0.1 units DNase, 0.6 units RNase inhibitor in TE) to remove contaminating DNA. The RNA was then extracted with phenol/chloroform/isoamyl, and ethanol precipitated. After quantitation at 260 nm, an aliquot was  
10 electrophoresed to check the integrity of the RNA. Next, Poly A<sup>+</sup> RNA was isolated using an Oligotex-dT kit from Qiagen (Chatsworth, CA) as described by the manufacturer. After quantitation, the mRNA was precipitated in ethanol and resuspended at a concentration of 1 mg/ml in water.  
15 Choroid plexus mRNA was used as a template for preparation of cDNA according to the method of Gubler et al. (*Gene* 25:263, 1983) using a Superscript Plasmid cDNA synthesis kit (Life Technologies; Gaithersburg, MD). The cDNA obtained was ligated into the NotI/Sal I sites of  
20 the mammalian expression vector pMET7, a modified version of pME18S, which utilizes the SRA promoter as described previously (Takebe, *Mol. Cell. Bio.* 8:466, 1988). Ligated cDNA was transformed into electrocompetent DH10B *E. coli* either prepared by standard procedures or  
25 obtained from Life Technologies.

#### DNA Preparation and Sequence Analysis

A cDNA clone from the murine choroid plexus library was sequenced to identify sequences of interest. The identified sequence was then used to clone and  
30 sequence a second murine splice variant of the *don-1* gene. The identification and analysis is performed as follows.

First, 96-well plates were inoculated with individual choroid plexus library transformants in 1 ml  
35 of LB-amp. These inoculations were based on the titers

- 63 -

of the cDNA transformants. The resulting cultures were grown for 15 to 16 hours at 37°C with aeration. Prior to DNA preparation, 100 ml of cell suspension was removed and added to 100 ml of 50% glycerol, mixed and stored at  
5 -80°C (glycerol freeze plate). DNA was then prepared using the Wizard miniprep system (Promega; Madison, WI) employing modifications for a 96-well format.

The insert cDNAs of a number of clones were sequenced by standard, automated fluorescent  
10 dideoxynucleotide sequencing using dye-primer chemistry (Applied Biosystems, Inc.; Foster City, CA) on Applied Biosystems 373 and 377 sequencers (Applied Biosystems). The primer used in this sequencing was proximal to the SRa promoter of the vector and therefore selective for  
15 the 5' end of the clones, although other primers with this selectivity can also be used. The short cDNA sequences obtained in this manner were screened as follows.

First, each sequence was checked to determine if  
20 it was a bacterial, ribosomal, or mitochondrial contaminant. Such sequences were excluded from the subsequent analysis. Second, sequence artifacts, such as vector and repetitive elements, were masked and/or removed from each sequence. Third, the remaining  
25 sequences were searched against a copy of the GenBank nucleotide database using the BLASTN program (BLASTN 1.3MP: Altschul et al., *J. Mol. Bio.* 215:403, 1990). Fourth, the sequences were analyzed against a non-redundant protein database with the BLASTX program  
30 (BLASTX 1.3MP: Altschul et al., *supra*). This protein database is a combination of the Swiss-Prot, PIR, and NCBI GenPept protein databases. The BLASTX program was run using the default BLOSUM-62 substitution matrix with the filter parameter: "xnu+seg". The score cutoff  
35 utilized was 75.

- 64 -

Assembly of overlapping clones into contigs was done using the program Sequencher (Gene Codes Corp.; Ann Arbor, MI). The assembled contigs were analyzed using the programs in the GCG package (Genetic Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711).

The above-described analysis resulted in the identification of a secreted, murine clone having an open reading frame of 139 amino acids. The protein encoded by this clone was named "murine Don-1." The amino-terminal portion of murine Don-1 has significant homology to the known heregulin gene. This portion is 41% identical to human heregulin based on a primary sequence alignment of the Ig and EGF domains of murine Don-1 with human heregulin.

This first splice variant of murine Don-1 was used as a probe to obtain an additional murine splice variant.

Splice variants of the human *don-1* gene were isolated in the same way from human fetal brain and fetal lung cDNA libraries (Clontech, Palo Alto, CA).

#### Example 2: Characterization of Don-1

The function of Don-1 polypeptide in a p185 assay and the expression pattern of Don-1 were examined as described below. Also described below is the expression of a recombinant form of soluble murine Don-1.

##### p185 Assay

MDA-MB453 cells (ATCC, Rockville, MD) were grown to 80% confluence in DMEM supplemented with 10% FCS in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. The cells were then replated in serum-free media for 24 hours before being exposed to NDF (100 ng/mL), EGF (100 ng/mL), or transfected 293Ebna-conditioned media (10%) for 15 minutes at 37°C. Cell lysates were prepared by

- 65 -

solubilizing cells in buffer (1% Triton X-100, 0.5% deoxycholate, 150 mM NaCl, 20 mM Tris pH 8.0, 1 mM EDTA, 30 mM  $\text{Na}_4\text{P}_2\text{O}_7$ , 50 mM NaF, 0.1 mM  $\text{Na}_3\text{VO}_4$ , 10  $\mu\text{g/mL}$  aprotinin, and 1 mM PMSF), and 100  $\mu\text{g}$  of protein was  
5 separated on a 10% SDS PAGE gel. Following transfer to nitrocellulose, immunodetection of phosphorylated proteins was performed using the monoclonal antiphosphotyrosine antibody 4G10 (Upstate Biotechnology, NY) as described by the manufacturer and utilizing  
10 Enhanced Chemiluminescence (ECL) (Amersham). NDF and EGF were purchased from R&D Systems (Minneapolis, MN).

Analysis of phosphorylated proteins by Western blotting revealed a robust induction of the 185 kDa protein in cells induced with NDF and in cells treated  
15 with Don-1 EGF-transfected 293Ebna cells. The level of induction seen with Don-1 EGF was comparable to saturating amounts of NDF and represented an approximate ten-fold increase in phosphorylation over uninduced cells. No induction of phosphorylation was observed in  
20 cells treated with EGF or the conditioned media of mock-transfected 293Ebna cells. This result demonstrates that Don-1 binds and activates a known member of the EGFR family, p185.

#### Analysis of Don-1 Expression

##### 25 Northern Analysis

Northern analysis was used to examine Don-1 expression as follows. Mouse and human multiple tissue northern blots purchased from Clontech (Palo Alto, Ca) were hybridized, according to manufacturer's directions,  
30 to a 1.4 kb Not/Sal fragment of murine Don-1 polypeptide SEQ ID NO:1, or to the 200 base-pair region encoding the EGF domain which extends from about amino acid location 104 to about amino acid location 140 of SEQ ID NO:1.

This Northern analysis revealed that Don-1 appears  
35 to be highly expressed in the mouse brain, although

- 66 -

multiple transcripts were also observed in the spleen and lung. The message is also differentially expressed throughout embryogenesis, indicating a possible role in development. In all positive tissues, multiple  
5 transcripts exist, the major sizes being about 4 kb and about 3 kb.

Human tissue Northern blots showed that human Don-1 is highly expressed in fetal brain and fetal lung tissues. In addition, two transcripts of about 4 kb and  
10 3 kb were detected exclusively in the cerebellum of human adult tissue. No other normal adult human tissues appeared to express human Don-1. However, Don-1 transcripts were detected in a human colon adenocarcinoma cell line SW480 and in a human melanoma cell line G361.  
15 In these tissues there were two major Don-1 transcripts of about 4.4 kb and about 3 kb each.

#### In Situ Analysis

*In situ* hybridizations were also used to examine Don-1 expression. Tissues for these hybridizations were  
20 prepared as follows. Four to six week old C57BL/6 mice were cervically dislocated, and their brains were removed and frozen on dry ice. Ten  $\mu$ m coronal frozen sections of brain were post-fixed with 4% formaldehyde in 1x phosphate buffered saline (PBS) (25°C) for 10 minutes,  
25 rinsed two times in 1x PBS, rinsed once in 1 M triethanolamine-HCl (pH 8), and then incubated in 0.25% acetic anhydride/1 M triethanolamine-HCl for 10 minutes. Sections were then rinsed in 2x SSC. Tissue was dehydrated through a series of ethanol washes, 70%  
30 ethanol for 1 minute, 80% for 1 minute, 95% for 2 minutes, and 100% ethanol for 1 minute. Sections were then incubated in 100% chloroform for 5 minutes and rinsed in 95% ethanol for 1 minute and 100% ethanol for 1 minute. Sections were air dried for 20 minutes.



- 67 -

Hybridizations were performed with <sup>35</sup>S-radiolabeled (5 x 10<sup>7</sup> cpm/ml) cRNA probes encoding a 472 bp segment of the 5' end of the murine Don-1 gene (SEQ ID NO:1, nucleotides 68-540). Probes were incubated in the presence of 600 mM NaCl, 10 mM Tris, pH 7.5, 1 mM EDTA, 0.01% sheared herring sperm, 0.01% yeast tRNA, 0.05% total yeast sRNA Type X1, 1x Denhardt's solution, 50% formamide, 10% dextran sulfate, 100 mM DTT, 0.1% SDS, and 0.1% Na thiosulfate for 18 hours at 55°C.

After hybridization, slides were washed with 2x SSC. Sections were then incubated with 10 mM Tris-HCl (pH 7.6)/500 mM NaCl/1 mM EDTA (TNE) at 37°C for 10 minutes, incubated in 10 µg/ml RNase A in TNE at 37° for 30 minutes, and washed in TNE at 37°C for 30 minutes. Sections were then rinsed with 2x SSC at room temperature, then incubated with 2x SSC at 50°C for 1 hour, rinsed and incubated with 0.2x SSC at 55°C for 1 hour, and then incubated with 0.2x SSC at 60°C for 1 hour. Sections were then dehydrated through a series of ethanols, 50%, 70%, 80%, and 90% with 0.3 M NH<sub>4</sub>OAc, and 100% ethanol. Sections were air dried and placed on Kodak Biomax MR scientific imaging film for 7 days at room temperature.

mRNA transcripts were localized to the cerebellum and Ammon's horn. Controls for the *in situ* hybridization experiments included the use of a sense probe which showed no signal above background levels and RNase treated tissue which showed a significantly reduced signal.

#### Expression Cloning

The EGF domain and flanking amino acids (amino acids 85-154 of SEQ ID NO:1) were amplified by PCR and then subcloned into a variety of commercially available bacterial expression vectors including pGEX (Pharmacia, Uppsala, Sweden), pMAL (NEB, Beverly, MA) and pTRX

- 68 -

(Invitrogen, San Diego, CA). Purification of recombinant material was performed as described by the manufacturer. This same domain was also subcloned into a mammalian expression vector, PN8E and then transfected into 293Ebna cells as detailed by Gibco-BRL (Gaithersburg, MD). A leader sequence (MALPVTALLLPLALLLHAARP; SEQ ID NO:24) was fused to the N-terminal of the EGF domain by PCR and a Flag epitope tag was placed on the C-terminal, prior to subcloning into PN8E (Ho et al., *P.N.A.S. USA*, **90**:11267-11271, 1993).

293Ebna cells at 80 percent confluence in 6-well dishes were transfected with 1.0  $\mu$ g DNA in 10  $\mu$ l lipofectamine (Gibco-BRL, Gaithersburg, MD) for 5 hours at 37°C in 5 percent CO<sub>2</sub> in an 800  $\mu$ l final volume. Following incubation, DMEM and 10 percent Fetal Calf Serum were added, and the media was replaced 24 hours after the start of transfection. Culture supernatant was collected 48 hours later.

#### Preparation of Soluble Don-1

Soluble forms of recombinant murine or human Don-1, or domains thereof, can be produced in bacteria using the pGEX expression system as described above for the EGF domain of SEQ ID NO:1. The pGEX-Don-1 is purified on glutathione agarose and the Don-1 moiety released by thrombin digestion. Following endotoxin removal on an Endotoxin BX column (Cape Cod Associates: Falmouth, MA) the Don-1 preparation is determined to contain low levels of endotoxin (<0.01 EU/ml) by the Limulus amoebocyte lysate (LAL) assay (Cape Cod Associates).

Recombinant, soluble Don-1 is produced as follows. First, the murine Don-1 cDNA is amplified with a primer corresponding to a sequence at the 5' end of the sequence encoding, for example, the EGF domain (5' primer). The 5' primer, 5'-AAAAAAGAATTCCTCCATGTCAACAGCGTG-3' (SEQ ID NO:25), has an EcoRI restriction enzyme cleavage site

- 69 -

followed by 18 nucleotides encoding the 5' flanking region of the EGF domain of murine Don-1. The 3' primer used was 5'-TCCTCTCTCGAGTCACTTAGGATCTGGCATGTA-3' (SEQ ID NO:26). This primer has complementary sequences encoding 5 amino acids 187 to 192 preceded by a termination codon and XhoI site.

These primer pairs were used for PCR amplification using the following conditions: 94°C for 30 seconds; 55°C for 30 seconds and 72°C for 90 seconds with 30 10 cycles. The resulting PCR product was cloned into the GST fusion protein vector pGEX (Pharmacia, Uppsala, Sweden). The fusion protein was produced in *E. coli* and purified according to the protocol supplied by the manufacturer. The Don-1 construct produced a protein of 15 approximately 7.0 kD after the cleavage of GST by thrombin.

#### Example 3: Mapping of the don-1 Gene

These examples describe chromosome mapping of the mouse and human don-1.

#### 20 Mouse Chromosome Mapping

The don-1 gene was mapped to the proximal end of chromosome 18 in the mouse, utilizing a *Mus spretus*/C57BL/6J backcross panel. Don-1 appears to be located close to cdc25, 17cM from the top of chromosome 25 18, between the markers D18Mit20 and D18Mit24.

PCR primers were used to amplify mouse genomic DNA using standard techniques. Primers were designed from noncoding sequences of murine don-1 and were as follows:

Forward primer: 5'-AGAGGAAGGCCAAAGTAGTG-3' (SEQ 30 ID NO:33), and

Reverse primer: 5'-GTGGACCACAAGGTAAACAG-3' (SEQ ID NO:34).

Other potential primers include:

- 70 -

Forward primer: 5'-CACAGTCCACCCCTCAG-3' (SEQ ID NO:27), and

Reverse primer: 5'-GCTCTGGTAAGCAAACATGG-3' (SEQ ID NO:28).

- 5 Amplification conditions were 30 cycles at 95°C for 1 minute, 60°C for 1 minute, and 72°C for 45 seconds. Samples were run on nondenaturing 10% acrylamide SSCP gel at 20 W and 4°C for 2.5 hours.

#### Human Chromosome Mapping

- 10 Human don-1 can be mapped to a particular chromosome by using a panel of radiation hybrids in a manner similar to that described for the mouse chromosome mapping.

- The following primers are used to amplify human  
15 genomic DNA from a panel of radiation hybrids (Genebridge 4, Research Genetics, Huntsville, AL):

Forward primer: 5'-TGTGAACTCCTCTGGCCTGT-3' (SEQ ID NO:29), and

- Reverse primer: 5'-GAAGGGGCTGGGCATTTAAT-3' (SEQ  
20 ID NO:30).

The amplification profile is as follows: 94°C for 30 seconds; 55°C for 30 seconds, and 72°C for 45 seconds with 30 cycles. Samples are resolved on 1% agarose TAE gel.

#### 25 Deposit of Microorganisms

The following microorganisms were deposited with the American Type Culture Collection (ATCC), Rockville, Maryland, on July 3, 1996 and assigned the indicated accession number:

30 <u>Microorganism</u>	<u>ATCC Accession</u>
<u>No.</u>	
<u>E. coli</u> CpmDon-1a (membrane-bound murine Don-1)	98096
<u>E. coli</u> CpmDon-1b (membrane-bound human Don-1)	98097

- 71 -

E. coli CpmDon-2 (secreted murine Don-1)

98098

Deposit Statement

The subject cultures have been deposited under conditions that assure that access to the cultures will  
5 be available during the pendency of the patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 USC 122. The deposits are available as required by foreign patent laws in countries wherein  
10 counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

15 Further, the subject culture deposits will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., they will be stored with all the care necessary to keep them viable and uncontaminated for  
20 a period of at least five years after the most recent request for the furnishing of a sample of the deposits, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the  
25 cultures plus five years after the last request for a sample from the deposit. The depositor acknowledges the duty to replace the deposits should the depository be unable to furnish a sample when requested, due to the condition of the deposits. All restrictions on the  
30 availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them.

- 72 -

Other Embodiments

The invention also features fragments, variants, analogs and derivatives of the Don-1 polypeptides described above that retain one or more of the biological  
5 activities of Don-1 such as activation of receptor-type tyrosine kinases as described herein.

The invention includes naturally-occurring and non-naturally-occurring allelic variants. Compared to the most common naturally-occurring nucleotide sequence  
10 encoding Don-1, the nucleic acid sequence encoding allelic variants may have a substitution, deletion, or addition of one or more nucleotides. The preferred allelic variants are functionally equivalent to naturally-occurring Don-1.

15 It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended  
20 claims. Other aspects, advantages, and modifications are within the scope of the following claims.

-73-

## SEQUENCE LISTING

## (1) GENERAL INFORMATION

- (i) APPLICANT: Millennium Biotherapeutics, Inc.
- (ii) TITLE OF THE INVENTION: DON-1 GENE AND POLYPEPTIDES AND USES THEREFOR
- (iii) NUMBER OF SEQUENCES: 33
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Fish & Richardson, P.C.
  - (B) STREET: 225 Franklin Street
  - (C) CITY: Boston
  - (D) STATE: MA
  - (E) COUNTRY: US
  - (F) ZIP: 02110-2804
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Diskette
  - (B) COMPUTER: IBM Compatible
  - (C) OPERATING SYSTEM: Windows95
  - (D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: US/PCT97/----
  - (B) FILING DATE: 18-AUG-1997
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 08/752,307
  - (B) FILING DATE: 19-NOV-1996
  - (A) APPLICATION NUMBER: 08/699,591
  - (B) FILING DATE: 19-AUG-1996
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Meiklejohn, Ph.D., Anita L.
  - (B) REGISTRATION NUMBER: 35,283
  - (C) REFERENCE/DOCKET NUMBER: 09404/022WO1
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 617-542-5070
  - (B) TELEFAX: 617-542-8906
  - (C) TELEX: 200154

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2467 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: circular
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
  - (A) NAME/KEY: Coding Sequence
  - (B) LOCATION: 79...1893

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

CCTAACGGCA AAAACATCAA GAAAGAGGTG GCAGAGATCC TGTGCACTGA CTGCGCCACC   60
CGGCCCAAGC TGAAGAAG ATG AAG AGC CAG ACA GGA GAG GTG GGT GAG AAG   111
Met Lys Ser Gln Thr Gly Glu Val Gly Glu Lys
      1             5             10

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-74-

CAG TCG CTC AAG TGT GAG GCA GCG GCG GGA AAC CCC CAG CCC TCC TAT Gln Ser Leu Lys Cys Glu Ala Ala Ala Gly Asn Pro Gln Pro Ser Tyr 15 20 25	159
CGC TGG TTC AAG GAT GGC AAG GAA CTC AAC CGG AGT CGT GAT ATT CGC Arg Trp Phe Lys Asp Gly Lys Glu Leu Asn Arg Ser Arg Asp Ile Arg 30 35 40	207
ATC AAG TAT GGC AAT GTC AGA AAG AAC TCA CGG CTA CAG TTC AAC AAA Ile Lys Tyr Gly Asn Val Arg Lys Asn Ser Arg Leu Gln Phe Asn Lys 45 50 55	255
GTG AGG GTG GAG GAT GCC GGG GAG TAC GTC TGT GAG GCC GAG AAC ATC Val Arg Val Glu Asp Ala Gly Glu Tyr Val Cys Glu Ala Glu Asn Ile 60 65 70 75	303
CTT GGG AAG GAC ACC GTG AGG GGC CGA CTC CAT GTC AAC AGC GTG AGC Leu Gly Lys Asp Thr Val Arg Gly Arg Leu His Val Asn Ser Val Ser 80 85 90	351
ACC ACT CTG TCA TCC TGG TCG GGA CAT GCC CGG AAG TGC AAT GAG ACC Thr Thr Leu Ser Ser Trp Ser Gly His Ala Arg Lys Cys Asn Glu Thr 95 100 105	399
GCC AAG TCC TAC TGT GTG AAT GGA GGC GTG TGC TAC TAC ATC GAG GGC Ala Lys Ser Tyr Cys Val Asn Gly Gly Val Cys Tyr Tyr Ile Glu Gly 110 115 120	447
ATC AAC CAG CTC TCC TGC AAA TGT CCA AAC GGA TTC TTC GGA CAG AGA Ile Asn Gln Leu Ser Cys Lys Cys Pro Asn Gly Phe Phe Gly Gln Arg 125 130 135	495
TGT TTG GAG AAA CTG CCT TTG CGA TTG TAC ATG CCA GAT CCT AAG CAA Cys Leu Glu Lys Leu Pro Leu Arg Leu Tyr Met Pro Asp Pro Lys Gln 140 145 150 155	543
AAG GCT GAG GAG CTG TAC CAG AAG AGA GTG CTG ACA ATT ACT GGT ATC Lys Ala Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile 160 165 170	591
TGT GTG GCC CTG CTG GTC GTG GGC ATC GTC TGT GTG GTC GCC TAC TGC Cys Val Ala Leu Leu Val Val Gly Ile Val Cys Val Val Ala Tyr Cys 175 180 185	639
AAG ACC AAA AAA CAG AGG AGG CAG ATG CAT CAT CAT CTC CGG CAG AAC Lys Thr Lys Lys Gln Arg Arg Gln Met His His His Leu Arg Gln Asn 190 195 200	687
ATG TGC CCA GCC CAC CAG AAC CGA AGC CTG GCC AAC GGG CCC AGC CAC Met Cys Pro Ala His Gln Asn Arg Ser Leu Ala Asn Gly Pro Ser His 205 210 215	735
CCT CGG CTG GAC CCT GAG GAG ATC CAG ATG GCA GAT TAC ATC TCC AAA Pro Arg Leu Asp Pro Glu Glu Ile Gln Met Ala Asp Tyr Ile Ser Lys 220 225 230 235	783
AAT GTG CCA GCT ACA GAC CAC GTG ATC CGG AGG GAA GCT GAG ACC ACG Asn Val Pro Ala Thr Asp His Val Ile Arg Arg Glu Ala Glu Thr Thr 240 245 250	831
TTC TCT GGG AGC CAC TCC TGT TCA CCT TCT CAC CAC TGC TCC ACA GCC Phe Ser Gly Ser His Ser Cys Ser Pro Ser His His Cys Ser Thr Ala 255 260 265	879
ACG CCC ACC TCC AGC CAC AGA CAT GAG AGC CAC ACG TGG AGC CTG GAA Thr Pro Thr Ser Ser His Arg His Glu Ser His Thr Trp Ser Leu Glu 270 275 280	927



-75-

CGT TCA GAG AGC CTG ACC TCG GAT TCC CAG TCA GGC ATC ATG CTA TCA Arg Ser Glu Ser Leu Thr Ser Asp Ser Gln Ser Gly Ile Met Leu Ser 285 290 295	975
TCA GTA GGC ACC AGC AAG TGC AAC AGC CCA GCA TGT GTG GAG GCA CGG Ser Val Gly Thr Ser Lys Cys Asn Ser Pro Ala Cys Val Glu Ala Arg 300 305 310 315	1023
GCG CGG AGG GCA GCA GCC TAC AGC CAG GAG GAG CGG CGC AGG GCT GCC Ala Arg Arg Ala Ala Tyr Ser Gln Glu Arg Arg Arg Ala Ala 320 325 330	1071
ATG CCA CCC TAC CAT GAC TCC ATA GAC TCG CTG CGT GAC TCT CCA CAC Met Pro Pro Tyr His Asp Ser Ile Asp Ser Leu Arg Asp Ser Pro His 335 340 345	1119
AGT GAA AGG TAC GTG TCA GCC TTG ACC ACG CCC GCT CGC CTC TCG CCC Ser Glu Arg Tyr Val Ser Ala Leu Thr Thr Pro Ala Arg Leu Ser Pro 350 355 360	1167
GTG GAC TTC CAC TAC TCG CTG GCC ACG CAG GTG CCG ACT TTC GAG ATC Val Asp Phe His Tyr Ser Leu Ala Thr Gln Val Pro Thr Phe Glu Ile 365 370 375	1215
ACG TCG CCC AAC TCT GAG CAT GCC GTG TCG CTG CCG CCC GCC GCG CCC Thr Ser Pro Asn Ser Glu His Ala Val Ser Leu Pro Pro Ala Ala Pro 380 385 390 395	1263
ATC AGC TAC CGC CTG GCG GAG CAG CAG CCG CTC CTG CGG CAT CCA GCG Ile Ser Tyr Arg Leu Ala Glu Gln Gln Pro Leu Leu Arg His Pro Ala 400 405 410	1311
CCG CCC GGC CCG GGG CCG GGG TCG GGG CCC GGA GCG GAC ATG CAG CGC Pro Pro Gly Pro Gly Pro Gly Ser Pro Gly Ala Asp Met Gln Arg 415 420 425	1359
AGC TAC GAC AGC TAC TAC TAC CCT GCG GCG GGG CCC GGG CCG CGG CGC Ser Tyr Asp Ser Tyr Tyr Tyr Pro Ala Ala Gly Pro Gly Pro Arg Arg 430 435 440	1407
AGC GCC TGC GCG CTG GGA GGC AGC TTG GGC AGC CTG CCC GCC AGC CCC Ser Ala Cys Ala Leu Gly Glu Ser Leu Gly Ser Leu Pro Ala Ser Pro 445 450 455	1455
TTC CGC ATC CCG GAG GAC GAC GAG TAC GAG ACC ACG CAG GAG TGC GCG Phe Arg Ile Pro Glu Asp Asp Glu Tyr Glu Thr Thr Gln Glu Cys Ala 460 465 470 475	1503
CCC CCG CCG CCG CCG CGG CCG CGC ACG CGC GGC GCG TCC CGC AGG ACG Pro Pro Pro Pro Pro Arg Pro Arg Thr Arg Gly Ala Ser Arg Arg Thr 480 485 490	1551
TCG GCG GGG CCG CGG CGC TGG CGG CGC TCC CGG CTC AAC GGG TTG GCG Ser Ala Gly Pro Arg Arg Trp Arg Arg Ser Arg Leu Asn Gly Leu Ala 495 500 505	1599
GCG CAG CGC GCA CGC GCG GCG CGG GAC TCG CTG TCA TTG AGC AGC GGT Ala Gln Arg Ala Arg Ala Ala Arg Asp Ser Leu Ser Leu Ser Ser Gly 510 515 520	1647
TCG GGC TGC GGC TCG GCG TCG GCC TCG GAC GAC GAC GCG GAC GAC GCG Ser Gly Cys Gly Ser Ala Ser Ala Ser Asp Asp Asp Ala Asp Asp Ala 525 530 535	1695
GAC GGG GCG CTG GCG GCC GAG AGC ACG CCA TTC CTC GGC CTG CGA GCG Asp Gly Ala Leu Ala Ala Glu Ser Thr Pro Phe Leu Gly Leu Arg Ala 540 545 550 555	1743

-76-

GCG CAC GAC GCG TTG CGC TCG GAC TCG CCG CCG CTG TGC CCC GCG GCC 1791  
 Ala His Asp Ala Leu Arg Ser Asp Ser Pro Pro Leu Cys Pro Ala Ala  
 560 565 570  
 GAC AGC AGG ACT TAC TAC TCC CTC GAC AGC CAC AGC ACG CGC GCC AGC 1839  
 Asp Ser Arg Thr Tyr Tyr Ser Leu Asp Ser His Ser Thr Arg Ala Ser  
 575 580 585  
 AGC AGA CAC AGC CGG GGG CCG CCC ACG AGG GCC AAG CAG GAC TCG GGG 1887  
 Ser Arg His Ser Arg Gly Pro Pro Thr Arg Ala Lys Gln Asp Ser Gly  
 590 595 600  
 CCC CTC TAAGGCCCCC CGCCTCGCCC GCCCCACGTC TCCAAGGAGA GCGGAGACCA CC 1945  
 Pro Leu  
 605  
 GACTGGAGAG GGAAAAGGAG CGAACAAAGA AATAAAAATA TTTTATTIT CTATAAAGG 2005  
 AAAAAAGTAT AACAAAATGT TTTATTTTCA TTTTAGCAAA AAAAATTGTC TTATAATACT 2065  
 AGCTAACGGC AAAGACGTTT TTATAGGGAA ACTATTTATA TGTAACATCC TGATTACAG 2125  
 CTTCCGAAAA AAAAAAGAA ACAACAAAAA AAAAAAAA AAAAATCGA GGGGGGGCCC 2185  
 GGTACCCAAT TCGCCCTATA GTGAGTCGTA TTACAATTCA CTGGCCGTCG TTTTACAACG 2245  
 TCGTGACTGG GAAACCCCTG GCGTTACCCA ACTTAATCGC CTTGCAGCAC ATCCCCCTTT 2305  
 CGCCAGCTGG CGTAATAGCG AAAAGGCCCG CACCGATCGC CCTTCCCAAC AGTTGCGCAG 2365  
 CCTGAATGCG GAATGCAAAA TTGTAAGCGT TAATATTTTG TTAAAATTCC CGTTAAATTT 2425  
 TTGTAAATC ACTCATTTT TAACCAATAG GCCGAAATCG GC 2467

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 605 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Ser Gln Thr Gly Glu Val Gly Glu Lys Gln Ser Leu Lys Cys  
 1 5 10 15  
 Glu Ala Ala Ala Gly Asn Pro Gln Pro Ser Tyr Arg Trp Phe Lys Asp  
 20 25 30  
 Gly Lys Glu Leu Asn Arg Ser Arg Asp Ile Arg Ile Lys Tyr Gly Asn  
 35 40 45  
 Val Arg Lys Asn Ser Arg Leu Gln Phe Asn Lys Val Arg Val Glu Asp  
 50 55 60  
 Ala Gly Glu Tyr Val Cys Glu Ala Glu Asn Ile Leu Gly Lys Asp Thr  
 65 70 75 80  
 Val Arg Gly Arg Leu His Val Asn Ser Val Ser Thr Thr Leu Ser Ser  
 85 90 95  
 Trp Ser Gly His Ala Arg Lys Cys Asn Glu Thr Ala Lys Ser Tyr Cys  
 100 105 110  
 Val Asn Gly Gly Val Cys Tyr Tyr Ile Glu Gly Ile Asn Gln Leu Ser  
 115 120 125  
 Cys Lys Cys Pro Asn Gly Phe Phe Gly Gln Arg Cys Leu Glu Lys Leu  
 130 135 140  
 Pro Leu Arg Leu Tyr Met Pro Asp Pro Lys Gln Lys Ala Glu Glu Leu  
 145 150 155 160  
 Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys Val Ala Leu Leu  
 165 170 175  
 Val Val Gly Ile Val Cys Val Val Ala Tyr Cys Lys Thr Lys Lys Gln  
 180 185 190  
 Arg Arg Gln Met His His His Leu Arg Gln Asn Met Cys Pro Ala His  
 195 200 205  
 Gln Asn Arg Ser Leu Ala Asn Gly Pro Ser His Pro Arg Leu Asp Pro  
 210 215 220  
 Glu Glu Ile Gln Met Ala Asp Tyr Ile Ser Lys Asn Val Pro Ala Thr  
 225 230 235 240

-77-

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Asp His Val Ile Arg Arg Glu Ala Glu Thr Thr Phe Ser Gly Ser His
      245      250      255
Ser Cys Ser Pro Ser His His Cys Ser Thr Ala Thr Pro Thr Ser Ser
      260      265      270
His Arg His Glu Ser His Thr Trp Ser Leu Glu Arg Ser Ser Leu
      275      280      285
Thr Ser Asp Ser Gln Ser Gly Ile Met Leu Ser Ser Val Gly Thr Ser
      290      295      300
Lys Cys Asn Ser Pro Ala Cys Val Glu Ala Arg Ala Arg Arg Ala Ala
      305      310      315      320
Ala Tyr Ser Gln Glu Arg Arg Arg Ala Ala Met Pro Pro Tyr His
      325      330      335
Asp Ser Ile Asp Ser Leu Arg Asp Ser Pro His Ser Glu Arg Tyr Val
      340      345      350
Ser Ala Leu Thr Thr Pro Ala Arg Leu Ser Pro Val Asp Phe His Tyr
      355      360      365
Ser Leu Ala Thr Gln Val Pro Thr Phe Glu Ile Thr Ser Pro Asn Ser
      370      375      380
Glu His Ala Val Ser Leu Pro Pro Ala Ala Pro Ile Ser Tyr Arg Leu
      385      390      395      400
Ala Glu Gln Gln Pro Leu Leu Arg His Pro Ala Pro Pro Gly Pro Gly
      405      410      415
Pro Gly Ser Gly Pro Gly Ala Asp Met Gln Arg Ser Tyr Asp Ser Tyr
      420      425      430
Tyr Tyr Pro Ala Ala Gly Pro Gly Pro Arg Arg Ser Ala Cys Ala Leu
      435      440      445
Gly Gly Ser Leu Gly Ser Leu Pro Ala Ser Pro Phe Arg Ile Pro Glu
      450      455      460
Asp Asp Glu Tyr Glu Thr Thr Gln Glu Cys Ala Pro Pro Pro Pro Pro
      465      470      475      480
Arg Pro Arg Thr Arg Gly Ala Ser Arg Arg Thr Ser Ala Gly Pro Arg
      485      490      495
Arg Trp Arg Arg Ser Arg Leu Asn Gly Leu Ala Ala Gln Arg Ala Arg
      500      505      510
Ala Ala Arg Asp Ser Leu Ser Leu Ser Ser Gly Ser Gly Cys Gly Ser
      515      520      525
Ala Ser Ala Ser Asp Asp Asp Ala Asp Asp Ala Asp Gly Ala Leu Ala
      530      535      540
Ala Glu Ser Thr Pro Phe Leu Gly Leu Arg Ala Ala His Asp Ala Leu
      545      550      555      560
Arg Ser Asp Ser Pro Leu Cys Pro Ala Ala Asp Ser Arg Thr Tyr
      565      570      575
Tyr Ser Leu Asp Ser His Ser Thr Arg Ala Ser Ser Arg His Ser Arg
      580      585      590
Gly Pro Pro Thr Arg Ala Lys Gln Asp Ser Gly Pro Leu
      595      600      605

```

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1607 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 79...621

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

CCTAACGGCA AAAACATCAA GAAAGAGGTG GGCAAGATCC TGTGCACTGA CTGCGCCACC 60
CGGCCCAAGC TGAAGAAG ATG AAG AGC CAG ACA GGA GAG GTG GGT GAG AAG 111
      Met Lys Ser Gln Thr Gly Glu Val Gly Glu Lys
        1                      5                      10

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-78-

CAG TCG CTC AAG TGT GAG GCA GCG GCG GGA AAC CCC CAG CCC TCC TAT	159
Gln Ser Leu Lys Cys Glu Ala Ala Gly Asn Pro Gln Pro Ser Tyr	
15 20 25	
CGC TGG TTC AAG GAT GGC AAG GAA CTC AAC CGG AGT CGT GAT ATT CGC	207
Arg Trp Phe Lys Asp Gly Lys Glu Leu Asn Arg Ser Arg Asp Ile Arg	
30 35 40	
ATC AAG TAT GGC AAT GTC AGA AAG AAC TCA CGG CTA CAG TTC AAC AAA	255
Ile Lys Tyr Gly Asn Val Arg Lys Asn Ser Arg Leu Gln Phe Asn Lys	
45 50 55	
GTG AGG GTG GAG GAT GCC GGG GAG TAC GTC TGT GAG GCC GAG AAC ATC	303
Val Arg Val Glu Asp Ala Gly Glu Tyr Val Cys Glu Ala Glu Asn Ile	
60 65 70 75	
CTT GGG AAG GAC ACC GTG AGG GGC CGA CTC CAT GTC AAC AGC GTG AGC	351
Leu Gly Lys Asp Thr Val Arg Gly Arg Leu His Val Asn Ser Val Ser	
80 85 90	
ACC ACT CTG TCA TCC TGG TCG GGA CAT GCC CGG AAG TGC AAT GAG ACC	399
Thr Thr Leu Ser Ser Trp Ser Gly His Ala Arg Lys Cys Asn Glu Thr	
95 100 105	
GCC AAG TCC TAC TGT GTG AAT GGA GGC GTG TGC TAC TAC ATC GAG GGC	447
Ala Lys Ser Tyr Cys Val Asn Gly Gly Val Cys Tyr Tyr Ile Glu Gly	
110 115 120	
ATC AAC CAG CTC TCC TGC AAA TGT CCA AAC GGA TTC TTC GGA CAG AGA	495
Ile Asn Gln Leu Ser Cys Lys Cys Pro Asn Gly Phe Phe Gly Gln Arg	
125 130 135	
TGT TTG GAG AAA CTG CCT TTG CGA TTG TAC ATG CCA GAT CCT AAG CAA	543
Cys Leu Glu Lys Leu Pro Leu Arg Leu Tyr Met Pro Asp Pro Lys Gln	
140 145 150 155	
AGT GTC CTG TGG GAT ACA CCG GGG ACA GGT GTC AGC AGT TCG CAA TGG	591
Ser Val Leu Trp Asp Thr Pro Gly Thr Gly Val Ser Ser Ser Gln Trp	
160 165 170	
TCA ACT TCT CCA AGC ACC TTG GAT TTG AAT TGAAGGAGGC TGAGGAGCTG TAC	644
Ser Thr Ser Pro Ser Thr Leu Asp Leu Asn	
175 180	
CAGAAGAGAG TGCTGACAAT TACTGGTATC TGTGTGGCCC TGCTGGTCGT GGGCATCGTC	704
TGTGTGGTCG CCTACTGCAA GACCAAAAAA CAGAGGAGGC AGATGCATCA TCATCTCCGG	764
CAGAACATGT GCCCAGCCCA CCAGAACC GA AGCCTGGCCA ACGGGCCAG CCACCTCGG	824
CTGGACCCCTG AGGAGATCCA GATGGCAGAT TACATCTCCA AAAATGTGCC AGCTACAGAC	884
CACGTGATCC GGAGGGAAGC TGAGACCACG TTCTCTGGGA GCCACTCCTG TTCACCTTCT	944
CACCACTGCT CCACAGCCAC GCCCACCTCC AGCCACAGAC ATGAGAGCCA CACGTGGAGC	1004
CTGGAAACGTT CAGAGAGCCT GACCTCGGAT TCCCAGTCAG GCATCATGCT ATCATCACTA	1064
GGCACCCAGCA AGTGCAACAG CCCAGCATGT GTGGAGGCAC GGGCGCGGAG GGCAGCAGCC	1124
TACAGCCAGG AGGAGCGGCG CAGGGCTGCC ATGCCACCTT ACCATGACTC CATAGACTCG	1184
CTGGGTGACT CTCCACACAG TGAAGGTAC GTGTCAGCCT TGACCAGGCC CGCTCGCCTC	1244
TGCGCCGTGG ACTTCCACTA CTCGCTGGCC ACGCAGGTGC CGACTTTCGA GATCAGTCG	1304
CCCAACTCTG CGCATGCCGT GTCGCTGCCG CCCGCCGCGC CCATCAGCTA CCGCCTGGCG	1364
GAGCAGCAGC CGCTCCTGCG GCATCCAGCG CCGCCCGGCC CCGGGCCGGG GTCGGGGCC	1424
GGAGCGGACA TGCAGCGCAG CTACGACAGC TACTACTACC CTGCGGCGGG GCCCGGGCCG	1484
CGGCGCAGCG CCTGCGCGCT GGGAGGCAGC TTGGGCAGCC TGCCCGCCAG CCCCTTCCGC	1544
ATCCCGGAGG ACGACGAGTA CGAGACCACG CAGGAGTGCG CGCCCCCGCC GCCCGCGCGG	1604
CCG	1607

## (2) INFORMATION FOR SEQ ID NO:4:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 181 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

-79-

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Lys Ser Gln Thr Gly Glu Val Gly Glu Lys Gln Ser Leu Lys Cys
 1           5           10           15
Glu Ala Ala Ala Gly Asn Pro Gln Pro Ser Tyr Arg Trp Phe Lys Asp
      20           25           30
Gly Lys Glu Leu Asn Arg Ser Arg Asp Ile Arg Ile Lys Tyr Gly Asn
 35           40           45
Val Arg Lys Asn Ser Arg Leu Gln Phe Asn Lys Val Arg Val Glu Asp
 50           55           60
Ala Gly Glu Tyr Val Cys Glu Ala Glu Asn Ile Leu Gly Lys Asp Thr
 65           70           75
Val Arg Gly Arg Leu His Val Asn Ser Val Ser Thr Thr Leu Ser Ser
      85           90           95
Trp Ser Gly His Ala Arg Lys Cys Asn Glu Thr Ala Lys Ser Tyr Cys
 100           105           110
Val Asn Gly Gly Val Cys Tyr Tyr Ile Glu Gly Ile Asn Gln Leu Ser
 115           120           125
Cys Lys Cys Pro Asn Gly Phe Phe Gly Gln Arg Cys Leu Glu Lys Leu
 130           135           140
Pro Leu Arg Leu Tyr Met Pro Asp Pro Lys Gln Ser Val Leu Trp Asp
 145           150           155
Thr Pro Gly Thr Gly Val Ser Ser Ser Gln Trp Ser Thr Ser Pro Ser
      165           170           175
Thr Leu Asp Leu Asn
      180

```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1884 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 664...1883

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

CAGCTACAGC GACAGCAGCA GCAGCAGCAG CGAGAGGAGC AGCAGCAGCA GCAGCAGCAG      60
CAGCGAGAGC GGCAGCAGCA GCAGGAGCAG CAGCAACAAC AGCAGCATCT CTCGTCCCGC      120
TGCCCCCA GAGCGCGGC CGCAGCAACA GCCGCAGCCC CGCAGCCCG CAGCCCGGAG      180
AGCGCGCGCC CGTTCGCGAG CCGCAGCCGC CGGCGGCATG AGGCGCGACC CGGCCCCCGG      240
CTTCTCCATG CTGCTCTTCG GTGTGTGCT CGCCTGCTAC TCGCCAGCC TCAAGTCAGT      300
GCAGGACCCG GCGTACAAGG CACCCGTGGT GGTGGAGGCC AAGGTACAGG GGCTGGTCCC      360
AGCGCGCGGC TCCAGCTCCA ACAGCACCCG AGAGCGGCC GCCTCGGGTC GGGTGGCGTT      420
GGTAAAGGTG CTGGACAAGT GGCCGCTCCG GAGCGGGGG CTGCAGCGCG AGCAGGTGAT      480
CAGCGTGGGC TCCTGTGTGC CGCTCGAAAG GAACAGCGC TACATCTTT TCCTGGAGCC      540
CACGGAACAG CCCTAGTCT TTAAGACGGC CTTGCCCCC CTGATACCAA CGGCAAAAT      600
CTCAAGAAAG AGGTGGGCAA GATCCTGTGC ACTGGCTGCG CCACCCGCC CAAGTTGAAG      660

AAG ATG AAG AGC CAG ACG GGA CAG GTG GGT GAG AAG CAA TCG CTG AAG      708
Met Lys Ser Gln Thr Gly Gln Val Gly Glu Lys Gln Ser Leu Lys
 1           5           10

TGT GAG GCA GCA GCC GGT AAT CCC CAG CCT TCC TAC CGT TGG TTC AAG      756
Cys Glu Ala Ala Ala Gly Asn Pro Gln Pro Ser Tyr Arg Trp Phe Lys
      20           25           30

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GAT Asp	GGC Gly	AAG Lys	GAG Glu 35	CTC Leu	AAC Asn	CGC Arg	AGC Ser	CGA Arg 40	GAC Asp	ATT Ile	CGC Arg	ATC Ile	AAA Lys 45	TAT Tyr	GGC Gly	804
AAC Asn	GGC Gly	AGA Arg 50	AAG Lys	AAC Asn	TCA Ser	CGA Arg	CTA Leu 55	CAG Gln	TTC Phe	AAC Asn	AAG Lys	GTG Val 60	AAG Lys	GTG Val	GAG Glu	852
GAC Asp	GCT Ala 65	GGG Gly	GAG Glu	TAT Tyr	GTC Val	TGC Cys 70	GAG Glu	GCC Ala	GAG Glu	AAC Asn	ATC Ile 75	CTG Leu	GGG Gly	AAG Lys	GAC Asp	900
ACC Thr 80	GTC Val	CGG Arg	GGC Gly	CGG Arg	CTT Leu 85	TAC Tyr	GTC Val	AAC Asn	AGC Ser	GTG Val 90	AGC Ser	ACC Thr	ACC Thr	CTG Leu	TCA Ser 95	948
TCC Ser	TGG Trp	TCC Ser	GGG Gly	CAC His 100	GCC Ala	CGG Arg	AAG Lys	TGC Cys	AAC Asn 105	GAG Glu	ACA Thr	GCC Ala	AAG Lys	TCC Ser 110	TAT Tyr	996
TGC Cys	GTC Val	AAT Asn 115	GGA Gly	GGC Gly	GTC Val	TGC Cys	TAC Tyr 120	TAC Tyr	ATC Ile	GAG Glu	GGC Gly	ATC Ile	AAC Asn 125	CAG Gln	CTC Leu	1044
TCC Ser	TGC Cys	AAA Lys 130	TGT Cys	CCA Pro	AAT Asn	GGA Gly 135	TTC Phe	TTC Phe	GGA Gly	CAG Gln	AGA Arg	TGT Cys 140	TTG Leu	GAG Glu	AAA Lys	1092
CTG Leu	CCT Pro 145	TTG Leu	CGA Arg	TTG Leu	TAC Tyr	ATG Met 150	CCA Pro	GAT Asp	CCT Pro	AAG Lys	CAA Gln 155	AAG Lys	CAC His	CTT Leu	GGA Gly	1140
TTT Leu 160	GAA Glu	TTA Leu	AAG Lys	GAA Glu	GCC Ala 165	GAG Glu	GAG Glu	CTG Leu	TAC Tyr	CAG Gln 170	AAG Lys	AGG Arg	GTC Val	CTG Leu 175	ACC Thr	1188
ATC Ile	ACG Thr	GGC Gly	ATC Ile	TGC Cys 180	GTG Val	GCT Ala	CTG Leu	CTG Leu	GTC Val 185	GTG Val	GGC Gly	ATC Ile	GTC Val	TGT Cys 190	GTG Val	1236
GTG Val	GCC Ala	TAC Tyr	TGC Cys 195	AAG Lys	ACC Thr	AAA Lys	AAA Lys	CAG Gln 200	CGG Arg	AAG Lys	CAG Gln	ATG Met	CAC His	AAC Asn	CAC His	1284
CTC Leu	CGG Arg	CAG Gln 210	AAC Asn	ATG Met	TGC Cys	CCG Pro	GCC Ala 215	CAT His	CAG Gln	AAC Asn	CGG Arg	AGC Ser 220	TTG Leu	GCC Ala	AAT Asn	1332
GGG Gly	CCC Pro 225	AGC Ser	CAC His	CCC Pro	CGG Arg	CTG Leu 230	GAC Asp	CCA Pro	GAG Glu	GAG Glu	ATC Ile 235	CAG Gln	ATG Met	GCA Ala	GAT Asp	1380
TAT Tyr 240	ATT Ile	TCC Ser	AAG Lys	AAC Asn	GTG Val 245	CCA Pro	GCC Ala	ACA Thr	GAC Asp	CAT His 250	GTC Val	ATC Ile	AGG Arg	AGA Arg	GAA Glu 255	1428
ACT Thr	GAG Glu	ACC Thr	ACC Thr	TTC Phe 260	TCT Ser	GGG Gly	AGC Ser	CAC His	TCC Ser 265	TGT Cys	TCT Ser	CCT Pro	TCT Ser	CAC His 270	CAC His	1476
TGC Cys	TCC Ser	ACA Thr	GCC Ala 275	ACA Thr	CCC Pro	ACC Thr	TCC Ser	AGC Ser 280	CAC His	AGA Arg	CAC His	GAG Glu	AGC Ser 285	CAC His	ACG Thr	1524
TGG Trp	AGC Ser	CTG Leu 290	GAA Glu	CGT Arg	TCT Ser	GAG Glu	AGC Ser 295	CTG Leu	ACT Thr	TCT Ser	GAC Asp	TCC Ser 300	CAG Gln	TCG Ser	GGG Gly	1572

-81-

ATC	ATG	CTA	TCA	TCA	GTG	GGT	ACC	AGC	AAA	TGC	AAC	AGC	CCA	GCA	TGT	1620
Ile	Met	Leu	Ser	Ser	Val	Gly	Thr	Ser	Lys	Cys	Asn	Ser	Pro	Ala	Cys	
	305					310					315					
GTG	GAG	GCC	CGG	GCA	AGG	CGG	GCA	GCA	GCC	TAC	AAC	CTG	GAG	GAG	CGG	1668
Val	Glu	Ala	Arg	Ala	Arg	Arg	Ala	Ala	Ala	Tyr	Asn	Leu	Glu	Glu	Arg	
	320				325					330					335	
CGC	AGG	GCC	ACC	GCG	CCA	CCC	TAT	CAC	GAT	TCC	GTG	GAC	TCC	CTT	CGC	1716
Arg	Arg	Ala	Thr		Pro	Pro	Tyr	His	Asp	Ser	Val	Asp	Ser	Leu	Arg	
			340						345					350		
GAC	TCC	CCA	CAC	AGC	GAG	AGG	TAC	GTG	TCG	GCC	CTG	ACC	ACG	CCC	GCG	1764
Asp	Ser	Pro	His	Ser	Glu	Arg	Tyr	Val	Ser	Ala	Leu	Thr	Thr	Pro	Ala	
			355				360						365			
CGC	CTC	TCG	CCC	GTG	GAC	TTC	CAC	TAC	TCG	CTG	GCC	ACG	CAG	GTG	CCA	1812
Arg	Leu	Ser	Pro	Val	Asp	Phe	His	Tyr	Ser	Leu	Ala	Thr	Gln	Val	Pro	
		370				375						380				
ACT	TTC	GAG	ATC	ACG	TCC	CCC	AAC	TCG	GCG	CAC	GCC	GTG	TCG	CTG	CCG	1860
Thr	Phe	Glu	Ile	Thr	Ser	Pro	Asn	Ser	Ala	His	Ala	Val	Ser	Leu	Pro	
	385					390					395					
CCG	GCG	GCG	CCC	ATC	AGT	TAC	CGC									1884
Pro	Ala	Ala	Pro	Ile	Ser	Tyr	Arg									
	400				405											

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 407 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met	Lys	Ser	Gln	Thr	Gly	Gln	Val	Gly	Glu	Lys	Gln	Ser	Leu	Lys	Cys	
1				5				10						15		
Glu	Ala	Ala	Ala	Gly	Asn	Pro	Gln	Pro	Ser	Tyr	Arg	Trp	Phe	Lys	Asp	
		20					25					30				
Gly	Lys	Glu	Leu	Asn	Arg	Ser	Arg	Asp	Ile	Arg	Ile	Lys	Tyr	Gly	Asn	
		35				40						45				
Gly	Arg	Lys	Asn	Ser	Arg	Leu	Gln	Phe	Asn	Lys	Val	Lys	Val	Glu	Asp	
	50				55						60					
Ala	Gly	Glu	Tyr	Val	Cys	Glu	Ala	Glu	Asn	Ile	Leu	Gly	Lys	Asp	Thr	
	65			70					75					80		
Val	Arg	Gly	Arg	Leu	Tyr	Val	Asn	Ser	Val	Ser	Thr	Thr	Leu	Ser	Ser	
		85						90					95			
Trp	Ser	Gly	His	Ala	Arg	Lys	Cys	Asn	Glu	Thr	Ala	Lys	Ser	Tyr	Cys	
	100							105					110			
Val	Asn	Gly	Gly	Val	Cys	Tyr	Tyr	Ile	Glu	Gly	Ile	Asn	Gln	Leu	Ser	
	115					120					125					
Cys	Lys	Cys	Pro	Asn	Gly	Phe	Phe	Gly	Gln	Arg	Cys	Leu	Glu	Lys	Leu	
	130				135						140					
Pro	Leu	Arg	Leu	Tyr	Met	Pro	Asp	Pro	Lys	Gln	Lys	His	Leu	Gly	Phe	
	145			150					155				160			
Glu	Leu	Lys	Glu	Ala	Glu	Glu	Leu	Tyr	Gln	Lys	Arg	Val	Leu	Thr	Ile	
		165						170					175			
Thr	Gly	Ile	Cys	Val	Ala	Leu	Leu	Val	Val	Gly	Ile	Val	Cys	Val	Val	
		180					185					190				
Ala	Tyr	Cys	Lys	Thr	Lys	Lys	Gln	Arg	Lys	Gln	Met	His	Asn	His	Leu	
	195					200					205					

-82-

```

Arg Gln Asn Met Cys Pro Ala His Gln Asn Arg Ser Leu Ala Asn Gly
210      215      220
Pro Ser His Pro Arg Leu Asp Pro Glu Glu Ile Gln Met Ala Asp Tyr
225      230      235
Ile Ser Lys Asn Val Pro Ala Thr Asp His Val Ile Arg Arg Glu Thr
245      250      255
Glu Thr Thr Phe Ser Gly Ser His Ser Cys Ser Pro Ser His His Cys
260      265      270
Ser Thr Ala Thr Pro Thr Ser Ser His Arg His Glu Ser His Thr Trp
275      280      285
Ser Leu Glu Arg Ser Glu Ser Leu Thr Ser Asp Ser Gln Ser Gly Ile
290      295      300
Met Leu Ser Ser Val Gly Thr Ser Lys Cys Asn Ser Pro Ala Cys Val
305      310      315
Glu Ala Arg Ala Arg Arg Ala Ala Tyr Asn Leu Glu Glu Arg Arg
325      330      335
Arg Ala Thr Ala Pro Pro Tyr His Asp Ser Val Asp Ser Leu Arg Asp
340      345      350
Ser Pro His Ser Glu Arg Tyr Val Ser Ala Leu Thr Thr Pro Ala Arg
355      360      365
Leu Ser Pro Val Asp Phe His Tyr Ser Leu Ala Thr Gln Val Pro Thr
370      375      380
Phe Glu Ile Thr Ser Pro Asn Ser Ala His Ala Val Ser Leu Pro Pro
385      390      395
Ala Ala Pro Ile Ser Tyr Arg
405

```

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1476 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 69...1475

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

CGGGCGGCGG GGGCGCAGCG CGGCAGCGGA GAGCTGAGGC CGTCCCACCG CCTGGGACCC      60
CGTGCAGA ATG TCG GAG TCC AGG AGG AGG GCC CGC GGC CGC GGC AAG AAG      110
Met Ser Glu Ser Arg Arg Arg Gly Arg Gly Arg Gly Lys Lys
1      5      10
CAC CCA GAG GGG AGG AAG CGG GAG AGG GAG CCC GAT CCC GGG GAG AAA      158
His Pro Glu Gly Arg Lys Arg Glu Arg Glu Pro Asp Pro Gly Glu Lys
15      20      25      30
GCC ACC CGG CCC AAG TTG AAG AAG ATG AAG AGC CAG ACG GGA CAG GTG      206
Ala Thr Arg Pro Lys Leu Lys Lys Met Lys Ser Gln Thr Gly Gln Val
35      40      45
GGT GAG AAG CAA TCG CTG AAG TGT GAG GCA GCA GCC GGT AAT CCC CAG      254
Gly Glu Lys Gln Ser Leu Lys Cys Glu Ala Ala Ala Gly Asn Pro Gln
50      55      60
CCT TCC TAC CGT TGG TTC AAG GAT GGC AAG GAG CTC AAC CGC AGC CGA      302
Pro Ser Tyr Arg Trp Phe Lys Asp Gly Lys Glu Leu Asn Arg Ser Arg
65      70      75
GAC ATT CGC ATC AAA TAT GGC AAC GGC AGA AAG AAC TCA CGA CTA CAG      350
Asp Ile Arg Ile Lys Tyr Gly Asn Gly Arg Lys Asn Ser Arg Leu Gln
80      85      90

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-83-

TTC AAC AAG GTG AAG GTG GAG GAC GCT GGG GAG TAT GTC TGC GAG GCC Phe Asn Lys Val Lys Val Glu Asp Ala Gly Tyr Val Cys Glu Ala 95 100 105 110	398
GAG AAC ATC CTG GGG AAG GAC ACC GTC CGG GGC CGG CTT TAC GTC AAC Glu Asn Ile Leu Gly Lys Asp Thr Val Arg Gly Arg Leu Tyr Val Asn 115 120 125	446
AGC GTG AGC ACC ACC CTG TCA TCC TGG TCG GGG CAC GCC CGG AAG TGC Ser Val Ser Thr Thr Leu Ser Ser Trp Ser Gly His Ala Arg Lys Cys 130 135 140	494
AAC GAG ACA GCC AAG TCC TAT TGC GTC AAT GGA GGC GTC TGC TAC TAC Asn Glu Thr Ala Lys Ser Tyr Cys Val Asn Gly Gly Val Cys Tyr Tyr 145 150 155	542
ATC GAG GGC ATC AAC CAG CTC TCC TGC AAA TGT CCA AAT GGA TTC TTC Ile Glu Gly Ile Asn Gln Leu Ser Cys Lys Cys Pro Asn Gly Phe Phe 160 165 170	590
GGA CAG AGA TGT TTG GAG AAA CTG CCT TTG CGA TTG TAC ATG CCA GAT Gly Gln Arg Cys Leu Glu Lys Leu Pro Leu Arg Leu Tyr Met Pro Asp 175 180 185 190	638
CCT AAG CAA AAA GCC GAG GAG CTG TAC CAG AAG AGG GTC CTG ACC ATC Pro Lys Gln Lys Ala Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile 195 200 205	686
ACG GGC ATC TGC GTG GCT CTG CTG GTC GTG GGC ATC GTC TGT GTG GTG Thr Gly Ile Cys Val Ala Leu Leu Val Val Gly Ile Val Cys Val Val 210 215 220	734
GCC TAC TGC AAG ACC AAA AAA CAG CGG AAG CAG ATG CAC AAC CAC CTC Ala Tyr Cys Lys Thr Lys Lys Gln Arg Lys Gln Met His Asn His Leu 225 230 235	782
CGG CAG AAC ATG TGC CCG GCC CAT CAG AAC CGG AGC TTG GCC AAT GGG Arg Gln Asn Met Cys Pro Ala His Gln Asn Arg Ser Leu Ala Asn Gly 240 245 250	830
CCC AGC CAC CCC CGG CTG GAC CCA GAG GAG ATC CAG ATG GCA GAT TAT Pro Ser His Pro Arg Leu Asp Pro Glu Glu Ile Gln Met Ala Asp Tyr 255 260 265 270	878
ATT TCC AAG AAC GTG CCA GCC ACA GAC CAT GTC ATC AGG AGA GAA ACT Ile Ser Lys Asn Val Pro Ala Thr Asp His Val Ile Arg Arg Glu Thr 275 280 285	926
GAG ACC ACC TTC TCT GGG AGC CAC TCC TGT TCT CCT TCT CAC CAC TGC Glu Thr Thr Phe Ser Gly Ser His Ser Cys Ser Pro Ser His His Cys 290 295 300	974
TCC ACA GCC ACA CCC ACC TCC AGC CAC AGA CAC GAG AGC CAC ACG TGG Ser Thr Ala Thr Pro Thr Ser Ser His Arg His Glu Ser His Thr Trp 305 310 315	1022
AGC CTG GAA CGT TCT GAG AGC CTG ACT TCT GAC TCC CAG TCG GGG ATC Ser Leu Glu Arg Ser Glu Ser Leu Thr Ser Asp Ser Gln Ser Gly Ile 320 325 330	1070
ATG CTA TCA TCA GTG GGT ACC AGC AAA TGC AAC AGC CCA GCA TGT GTG Met Leu Ser Ser Val Gly Thr Ser Lys Cys Asn Ser Pro Ala Cys Val 335 340 345 350	1118
GAG GCC CGG GCA AGG CGG GCA GCA GCC TAC AAC CTG GAG GAG CGG CGC Glu Ala Arg Ala Arg Arg Ala Ala Tyr Asn Leu Glu Glu Arg Arg 355 360 365	1166

-84-

AGG GCC ACC GCG CCA CCC TAT CAC GAT TCC GTG GAC TCC CTT CGC GAC	1214
Arg Ala Thr Ala Pro Pro Tyr His Asp Ser Val Asp Ser Leu Arg Asp	
370 375 380	
TCC CCA CAC AGC GAG AGG TAC GTG TCG GCC CTG ACC ACG CCC GCG CGC	1262
Ser Pro His Ser Glu Arg Tyr Val Ser Ala Leu Thr Thr Pro Ala Arg	
385 390 395	
CTC TCG CCC GTG GAC TTC CAC TAC TCG CTG GCC ACG CAG GTG CCA ACT	1310
Leu Ser Pro Val Asp Phe His Tyr Ser Leu Ala Thr Gln Val Pro Thr	
400 405 410	
TTC GAG ATC ACG TCC CCC AAC TCG GCG CAC GCC GTG TCG CTG CCG CCG	1358
Phe Glu Ile Thr Ser Pro Asn Ser Ala His Ala Val Ser Leu Pro Pro	
415 420 425 430	
GCG GCG CCC ATC AGT TAC CGC CTG GCC CAG CAG CAG CCG TTA CTG CCG	1406
Ala Ala Pro Ile Ser Tyr Arg Leu Ala Glu Gln Gln Pro Leu Leu Arg	
435 440 445	
CAC CCG GCG CCC CCC GGC CCG GGA CCC GGA CCC GGG CCC GGG CCC GGG	1454
His Pro Ala Pro Pro Gly Pro Gly Pro Gly Pro Gly Pro Gly Pro Gly	
450 455 460	
CCC GGC GCA GAC ACC GGA ATT C	1476
Pro Gly Ala Asp Thr Gly Ile	
465	

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 469 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Ser	Glu	Ser	Arg	Arg	Arg	Gly	Arg	Gly	Arg	Gly	Lys	Lys	His	Pro
1				5				10						15	
Glu	Gly	Arg	Lys	Arg	Glu	Arg	Glu	Pro	Asp	Pro	Gly	Glu	Lys	Ala	Thr
			20					25					30		
Arg	Pro	Lys	Leu	Lys	Lys	Met	Lys	Ser	Gln	Thr	Gly	Gln	Val	Gly	Glu
		35				40						45			
Lys	Gln	Ser	Leu	Lys	Cys	Glu	Ala	Ala	Ala	Gly	Asn	Pro	Gln	Pro	Ser
		50			55					60					
Tyr	Arg	Trp	Phe	Lys	Asp	Gly	Lys	Glu	Leu	Asn	Arg	Ser	Arg	Asp	Ile
		65			70					75				80	
Arg	Ile	Lys	Tyr	Gly	Asn	Gly	Arg	Lys	Asn	Ser	Arg	Leu	Gln	Phe	Asn
			85						90					95	
Lys	Val	Lys	Val	Glu	Asp	Ala	Gly	Glu	Tyr	Val	Cys	Glu	Ala	Glu	Asn
			100					105					110		
Ile	Leu	Gly	Lys	Asp	Thr	Val	Arg	Gly	Arg	Leu	Tyr	Val	Asn	Ser	Val
		115				120						125			
Ser	Thr	Thr	Leu	Ser	Ser	Trp	Ser	Gly	His	Ala	Arg	Lys	Cys	Asn	Glu
		130				135					140				
Thr	Ala	Lys	Ser	Tyr	Cys	Val	Asn	Gly	Gly	Val	Cys	Tyr	Tyr	Ile	Glu
		145			150					155				160	
Gly	Ile	Asn	Gln	Leu	Ser	Cys	Lys	Cys	Pro	Asn	Gly	Phe	Phe	Gly	Gln
			165						170					175	
Arg	Cys	Leu	Glu	Lys	Leu	Pro	Leu	Arg	Leu	Tyr	Met	Pro	Asp	Pro	Lys
		180						185					190		
Gln	Lys	Ala	Glu	Glu	Leu	Tyr	Gln	Lys	Arg	Val	Leu	Thr	Ile	Thr	Gly
		195				200						205			

-85-

```

Ile Cys Val Ala Leu Leu Val Val Gly Ile Val Cys Val Val Ala Tyr
210      215
Cys Lys Thr Lys Lys Gln Arg Lys Gln Met His Asn His Leu Arg Gln
225      230      235      240
Asn Met Cys Pro Ala His Gln Asn Arg Ser Leu Ala Asn Gly Pro Ser
245      250      255
His Pro Arg Leu Asp Pro Glu Glu Ile Gln Met Ala Asp Tyr Ile Ser
260      265      270
Lys Asn Val Pro Ala Thr Asp His Val Ile Arg Arg Glu Thr Glu Thr
275      280      285
Thr Phe Ser Gly Ser His Ser Cys Ser Pro Ser His His Cys Ser Thr
290      295      300
Ala Thr Pro Thr Ser Ser His Arg His Glu Ser His Thr Trp Ser Leu
305      310      315      320
Glu Arg Ser Glu Ser Leu Thr Ser Asp Ser Gln Ser Gly Ile Met Leu
325      330      335
Ser Ser Val Gly Thr Ser Lys Cys Asn Ser Pro Ala Cys Val Glu Ala
340      345      350
Arg Ala Arg Arg Ala Ala Ala Tyr Asn Leu Glu Glu Arg Arg Ala
355      360      365
Thr Ala Pro Pro Tyr His Asp Ser Val Asp Ser Leu Arg Asp Ser Pro
370      375      380
His Ser Glu Arg Tyr Val Ser Ala Leu Thr Thr Pro Ala Arg Leu Ser
385      390      395      400
Pro Val Asp Phe His Tyr Ser Leu Ala Thr Gln Val Pro Thr Phe Glu
405      410      415
Ile Thr Ser Pro Asn Ser Ala His Ala Val Ser Leu Pro Pro Ala Ala
420      425      430
Pro Ile Ser Tyr Arg Leu Ala Glu Gln Gln Pro Leu Leu Arg His Pro
435      440      445
Ala Pro Pro Gly Pro Gly Pro Gly Pro Gly Pro Gly Pro Gly
450      455      460
Ala Asp Thr Gly Ile
465

```

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 422 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

Met Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys Lys Lys
1      5      10      15
Asp Arg Gly Ser Arg Gly Lys Pro Gly Pro Ala Glu Gly Asp Pro Ser
20      25      30
Pro Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu Ser Ala
35      40      45
Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser
50      55      60
Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu Leu Asn Arg Lys
65      70      75      80
Asn Lys Pro Glu Asn Ile Lys Ile Gln Lys Lys Pro Gly Lys Ser Glu
85      90      95
Leu Arg Ile Asn Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys
100      105      110
Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr
115      120      125
Ile Val Glu Ser Asn Glu Phe Ile Thr Gly Met Pro Ala Ser Thr Glu
130      135      140
Thr Ala Tyr Val Ser Ser Glu Ser Pro Ile Arg Ile Ser Val Ser Thr
145      150      155      160
Glu Gly Ala Asn Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr
165      170      175

```

-86-

```

Ser His Leu Ile Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
      180      185      190
Gly Gly Glu Cys Phe Thr Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
      195      200      205
Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn
      210      215      220
Val Pro Met Lys Val Gln Thr Gln Glu Lys Ala Glu Glu Leu Tyr Gln
      225      230      235      240
Lys Arg Val Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu Val Val
      245      250      255
Gly Ile Met Cys Val Val Ala Tyr Cys Lys Thr Lys Lys Gln Arg Gln
      260      265      270
Lys Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg Ser Asn
      275      280      285
Leu Val Asn Ile Ala Asn Gly Pro His His Pro Asn Pro Pro Pro Glu
      290      295      300
Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn Val Ile Ser Ser
      305      310      315      320
Glu His Ile Val Glu Arg Glu Val Glu Thr Ser Phe Ser Thr Ser His
      325      330      335
Tyr Thr Ser Thr Ala His His Ser Thr Thr Val Thr Gln Thr Pro Ser
      340      345      350
His Ser Trp Ser Asn Gly His Thr Glu Ser Val Ile Ser Glu Ser Asn
      355      360      365
Ser Val Ile Met Met Ser Ser Val Glu Asn Ser Arg His Ser Ser Pro
      370      375      380
Ala Gly Gly Pro Arg Gly Arg Leu His Gly Leu Gly Gly Pro Arg Asp
      385      390      395      400
Asn Ser Phe Leu Arg His Ala Arg Glu Thr Pro Asp Ser Tyr Arg Asp
      405      410      415
Ser Pro His Ser Glu Arg
      420

```

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 645 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

Met Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys Lys
  1      5      10      15
Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser Gln Ser
      20      25      30
Pro Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu Ser Ala
      35      40      45
Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser
      50      55      60
Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu Leu Asn Arg Lys
      65      70      75      80
Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys Lys Pro Gly Lys Ser Glu
      85      90      95
Leu Arg Ile Asn Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys
      100      105      110
Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr
      115      120      125
Ile Val Glu Ser Asn Glu Ile Ile Thr Gly Met Pro Ala Ser Thr Glu
      130      135      140
Gly Ala Tyr Val Ser Ser Glu Ser Pro Ile Arg Ile Ser Val Ser Thr
      145      150      155      160
Glu Gly Ala Asn Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr
      165      170      175
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
      180      185      190

```

-87-

Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr  
 195 200 205  
 Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr  
 210 215 220  
 Val Met Ala Ser Phe Tyr Lys His Leu Gly Ile Glu Phe Met Glu Ala  
 225 230 235 240  
 Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys Ile  
 245 250 255  
 Ala Leu Leu Val Val Gly Ile Met Cys Val Val Ala Tyr Cys Lys Thr  
 260 265 270  
 Lys Lys Gln Arg Lys Lys Leu His Asp Arg Leu Arg Gln Ser Leu Arg  
 275 280 285  
 Ser Glu Arg Asn Asn Met Met Asn Ile Ala Asn Gly Pro His His Pro  
 290 295 300  
 Asn Pro Pro Pro Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys  
 305 310 315 320  
 Asn Val Ile Ser Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu Thr Ser  
 325 330 335  
 Phe Ser Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr Thr Val  
 340 345 350  
 Thr Gln Thr Pro Ser His Ser Trp Ser Asn Gly His Thr Glu Ser Ile  
 355 360 365  
 Leu Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val Glu Asn Ser  
 370 375 380  
 Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn Gly Thr  
 385 390 395 400  
 Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg His Ala Arg Glu Thr  
 405 410 415  
 Pro Asp Ser Tyr Arg Asp Ser Pro His Ser Glu Arg Tyr Val Ser Ala  
 420 425 430  
 Met Thr Thr Pro Ala Arg Met Ser Pro Val Asp Phe His Thr Pro Ser  
 435 440 445  
 Ser Pro Lys Ser Pro Pro Ser Glu Met Ser Pro Pro Val Ser Ser Met  
 450 455 460  
 Thr Val Ser Met Pro Ser Met Ala Val Ser Pro Phe Met Glu Glu Glu  
 465 470 475 480  
 Arg Pro Leu Leu Leu Val Thr Pro Pro Arg Leu Arg Glu Lys Lys Phe  
 485 490 495  
 Asp His His Pro Gln Gln Phe Ser Ser Phe His His Asn Pro Ala His  
 500 505 510  
 Asp Ser Asn Ser Leu Pro Ala Ser Pro Leu Arg Ile Val Glu Asp Glu  
 515 520 525  
 Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro Ala Gln Glu Pro Val Lys  
 530 535 540  
 Lys Leu Ala Asn Ser Arg Arg Ala Lys Arg Thr Lys Pro Asn Gly His  
 545 550 555 560  
 Ile Ala Asn Arg Leu Glu Val Asp Ser Asn Thr Ser Ser Gln Ser Ser  
 565 570 575  
 Asn Ser Glu Ser Glu Thr Glu Asp Glu Arg Val Gly Glu Asp Thr Pro  
 580 585 590  
 Phe Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser Leu Glu Ala Thr Pro  
 595 600 605  
 Ala Phe Arg Leu Ala Asp Ser Arg Thr Asn Pro Ala Gly Arg Phe Ser  
 610 615 620  
 Thr Gln Glu Glu Ile Gln Ala Arg Leu Ser Ser Val Ile Ala Asn Gln  
 625 630 635 640  
 Asp Pro Ile Ala Val  
 645

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

-88-

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```

Gly His Ala Arg Lys Cys Asn Glu Thr Ala Lys Ser Tyr Cys Val Asn
 1           5           10           15
Gly Gly Val Cys Tyr Tyr Ile Glu Gly Ile Asn Gln Leu Ser Cys Lys
          20           25           30
Cys Pro Asn Gly Phe Phe Gly Gln Arg Cys Leu Glu Lys Leu Pro
          35           40           45

```

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
 1           5           10           15
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
          20           25           30
Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn
          35           40           45
Val Pro
          50

```

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```

Ser His Leu Ile Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
 1           5           10           15
Gly Gly Glu Cys Phe Thr Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
          20           25           30
Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn
          35           40           45
Val Pro
          50

```

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```

Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
 1           5           10           15
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
          20           25           30
Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr
          35           40           45

```

-89-

Val Met  
50

## (2) INFORMATION FOR SEQ ID NO:15:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```

Ser His Leu Thr Lys Cys Asp Ile Lys Gln Lys Ala Phe Cys Val Asn
 1           5           10           15
Gly Gly Glu Cys Tyr Met Val Lys Asp Leu Pro Asn Pro Pro Arg Tyr
      20           25           30
Leu Cys Arg Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr
      35           40           45
Val Met
50

```

## (2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```

Gly Lys Arg Asp Pro Cys Leu Arg Lys Tyr Lys Asp Phe Cys Ile His
 1           5           10           15
Gly Glu Cys Lys Tyr Val Lys Glu Leu Arg Ala Pro Ser Cys Ile Cys
      20           25           30
His Pro Gly Tyr His Gly Glu Arg Cys His Gly Leu Ser Leu
      35           40           45

```

## (2) INFORMATION FOR SEQ ID NO:17:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```

Asn Ser Asp Ser Glu Cys Pro Leu Ser His Asp Gly Tyr Cys Leu His
 1           5           10           15
Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn
      20           25           30
Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu
      35           40           45

```

## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids

-90-

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```

Lys Lys Lys Asn Pro Cys Asn Ala Glu Phe Gln Asn Phe Cys Ile His
 1           5           10           15
Gly Glu Cys Lys Tyr Ile Glu His Leu Glu Ala Val Thr Cys Lys Cys
          20           25           30
Gln Gln Glu Tyr Phe Gly Glu Arg Cys Gly Glu Lys Ser Met
      35           40           45

```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```

Ser His Phe Asn Asp Cys Pro Asp Ser His Thr Gln Phe Cys Phe His
 1           5           10           15
Gly Thr Cys Arg Phe Leu Val Gln Glu Asp Lys Pro Ala Cys Val Cys
          20           25           30
His Ser Gly Tyr Val Gly Ala Arg Cys Glu His Ala Asp Leu
      35           40           45

```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```

Val Leu Thr Ile Thr Gly Ile Cys Val Ala Leu Leu Val Val Gly Ile
 1           5           10           15
Val Cys Val Val Ala Tyr Cys
          20

```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 9...9

(D) OTHER INFORMATION: where Xaa at position 9 is  
Isoleucine or Valine

(A) NAME/KEY: Other

(B) LOCATION: 17...17

(D) OTHER INFORMATION: where Xaa at position 17 is  
Methionine or Valine



-91-

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Val Leu Thr Ile Thr Gly Ile Cys Xaa Ala Leu Leu Val Val Gly Ile  
 1 5 10 15  
 Xaa Cys Val Val Ala Tyr Cys  
 20

## (2) INFORMATION FOR SEQ ID NO:22:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GACTTGGCTC TCTCG

15

## (2) INFORMATION FOR SEQ ID NO:23:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGACTCCGAC ATTCT

15

## (2) INFORMATION FOR SEQ ID NO:24:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu  
 1 5 10 15  
 His Ala Ala Arg Pro  
 20

## (2) INFORMATION FOR SEQ ID NO:25:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AAAAAGAATT CCTCCATGTC AACAGCGTG

29

## (2) INFORMATION FOR SEQ ID NO:26:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid

-92-

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TCCTCTCTCG AGTCACTTAG GATCTGGCAT GTA

33

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CACAGTCCAC CCCTCAG

17

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GCTCTGGTAA GCAAACATGG

20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TGTGAACTCC TCTGGCCTGT

20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GAAGGGGCTG GGCATTTAAT

20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2268 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-93-

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 69...2009

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```

CGGGCGGGCGG GGGCGCAGCG CGGCAGCGGA GAGCTGAGGC CGTCCCACCG CCTGGGACCC      60
CGTGCAGA ATG TCG GAG TCC AAG AGG AGG GGC CGC GGC CGC GGC AAG AAG      110
Met Ser Glu Ser Lys Arg Arg Gly Arg Gly Arg Gly Lys Lys
      1              5              10

CAC CCA GAG GGG AGG AAG CGG GAG AGG GAG CCC GAT CCC GGG GAG AAA      158
His Pro Glu Gly Arg Lys Arg Glu Arg Glu Pro Asp Pro Gly Glu Lys
      15              20              25              30

GCC ACC CGG CCC AAG TTG AAG AAG ATG AAG AGC CAG ACG GGA CAG GTG      206
Ala Thr Arg Pro Lys Leu Lys Lys Met Lys Ser Gln Thr Gly Gln Val
      35              40              45

GGT GAG AAG CAA TCG CTG AAG TGT GAG GCA GCA GCC GGT AAT CCC CAG      254
Gly Glu Lys Gln Ser Leu Lys Cys Glu Ala Ala Ala Gly Asn Pro Gln
      50              55              60

CCT TCC TAC CGT TGG TTC AAG GAT GGC AAG GAG CTC AAC CGC AGC CGA      302
Pro Ser Tyr Arg Trp Phe Lys Asp Gly Lys Glu Leu Asn Arg Ser Arg
      65              70              75

GAC ATT CGC ATC AAA TAT GGC AAC GGC AGA AAG AAC TCA CGA CTA CAG      350
Asp Ile Arg Ile Lys Tyr Gly Asn Gly Arg Lys Asn Ser Arg Leu Gln
      80              85              90

TTC AAC AAG GTG AAG GTG GAG GAC GCT GGG GAG TAT GTC TGC GAG GCC      398
Phe Asn Lys Val Lys Val Glu Asp Ala Gly Glu Tyr Val Cys Glu Ala
      95              100              105              110

GAG AAC ATC CTG GGG AAG GAC ACC GTC CGG GGC CGG CTT TAC GTC AAC      446
Glu Asn Ile Leu Gly Lys Asp Thr Val Arg Gly Arg Leu Tyr Val Asn
      115              120              125

AGC GTG AGC ACC ACC CTG TCA TCC TGG TCG GGG CAC GCC CGG AAG TGC      494
Ser Val Ser Thr Thr Leu Ser Ser Trp Ser Gly His Ala Arg Lys Cys
      130              135              140

AAC GAG ACA GCC AAG TCC TAT TGC GTC AAT GGA GGC GTC TGC TAC TAC      542
Asn Glu Thr Ala Lys Ser Tyr Cys Val Asn Gly Gly Val Cys Tyr Tyr
      145              150              155

ATC GAG GGC ATC AAC CAG CTC TCC TGC AAA TGT CCA AAT GGA TTC TTC      590
Ile Glu Gly Ile Asn Gln Leu Ser Cys Lys Cys Pro Asn Gly Phe Phe
      160              165              170

GGA CAG AGA TGT TTG GAG AAA CTG CCT TTG CGA TTG TAC ATG CCA GAT      638
Gly Gln Arg Cys Leu Glu Lys Leu Pro Leu Arg Leu Tyr Met Pro Asp
      175              180              185              190

CCT AAG CAA AAA GCC GAG GAG CTG TAC CAG AAG AGG GTC CTG ACC ATC      686
Pro Lys Gln Lys Ala Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile
      195              200              205

ACG GGC ATC TGC GTG GCT CTG CTG GTC GTG GGC ATC GTC TGT GTG GTG      734
Thr Gly Ile Cys Val Ala Leu Leu Val Val Gly Ile Val Cys Val Val
      210              215              220

GCC TAC TGC AAG ACC AAA AAA CAG CGG AAG CAG ATG CAC AAC CAC CTC      782
Ala Tyr Cys Lys Thr Lys Lys Gln Arg Lys Gln Met His Asn His Leu
      225              230              235

```

-94-

CGG Arg	CAG Gln	AAC Asn	ATG Met	TGC Cys	CCG Pro	GCC Ala	CAT His	CAG Gln	AAC Asn	CGG Arg	AGC Ser	TTG Leu	GCC Ala	AAT Asn	GGG Gly	830
240	240					245				250						
CCC Pro	AGC Ser	CAC His	CCC Pro	CGG Arg	CTG Leu	GAC Asp	CCA Pro	GAG Glu	GAG Glu	ATG Met	CAG Gln	ATG Met	GCA Ala	GAT Asp	TAT Tyr	878
255					260					265					270	
ATT Ile	TCC Ser	AAG Lys	AAC Asn	GTG Val	CCA Pro	GCC Ala	ACA Thr	GAC Asp	CAT His	GTC Val	ATC Ile	AGG Arg	AGA Arg	GAA Glu	ACT Thr	926
				275				280						285		
GAG Glu	ACC Thr	ACC Thr	TTC Phe	TCT Ser	GGG Gly	AGC Ser	CAC His	TCC Ser	TGT Cys	TCT Ser	CCT Pro	TCT Ser	CAC His	CAC His	TGC Cys	974
			290					295					300			
TCC Ser	ACA Thr	GCC Ala	ACA Thr	CCC Pro	ACC Thr	TCC Ser	AGC Thr	CAC His	AGA Arg	CAC His	GAG Glu	AGC Ser	CAC His	ACG Thr	TGG Trp	1022
		305					310					315				
AGC Ser	CTG Leu	GAA Glu	CGT Arg	TCT Ser	GAG Glu	AGC Ser	CTG Leu	ACT Thr	TCT Ser	GAC Asp	TCC Ser	CAG Gln	TCG Ser	GGG Gly	ATC Ile	1070
	320					325				330						
ATG Met	CTA Leu	TCA Ser	TCA Ser	GTG Val	GGT Gly	ACC Thr	AGC Ser	AAA Lys	TGC Cys	AAC Asn	AGC Ser	CCA Pro	GCA Ala	TGT Cys	GTG Val	1118
335					340					345					350	
GAG Glu	GCC Ala	CGG Arg	GCA Ala	AGG Arg	CGG Arg	GCA Ala	GCA Ala	GCC Ala	TAC Tyr	AAC Asn	CTG Leu	GAG Glu	GAG Glu	CGG Arg	CGC Arg	1166
				355				360					365			
AGG Arg	GCC Ala	ACC Thr	GCG Ala	CCA Pro	CCC Pro	TAT Tyr	CAC His	GAT Asp	TCC Ser	GTG Val	GAC Asp	TCC Ser	CTT Leu	CGC Arg	GAC Asp	1214
			370					375					380			
TCC Ser	CCA Pro	CAC His	AGC Ser	GAG Glu	AGG Arg	TAC Tyr	GTG Val	TCG Ser	GCC Ala	CTG Leu	ACC Thr	ACG Thr	CCC Pro	GCG Ala	CGC Arg	1262
		385					390					395				
CTC Leu	TCG Ser	CCC Pro	GTG Val	GAC Asp	TTC Phe	CAC His	TAC Tyr	TCG Ser	CTG Leu	GCC Ala	ACG Thr	CAG Gln	GTG Val	CCA Pro	ACT Thr	1310
	400				405					410						
TTC Phe	GAG Glu	ATC Ile	ACG Thr	TCC Ser	CCC Pro	AAC Asn	TCG Ser	GCG Ala	CAC His	GCC Ala	GTG Val	TCG Ser	CTG Leu	CCG Pro	CCG Pro	1358
415					420				425						430	
GCG Ala	GCG Ala	CCC Pro	ATC Ile	AGT Ser	TAC Tyr	CGC Arg	CTG Leu	GCC Ala	GAG Glu	CAG Gln	CAG Gln	CCG Pro	TTA Leu	CTG Leu	CGG Arg	1406
				435				440					445			
CAC His	CCG Pro	GCG Ala	CCC Pro	CCC Pro	GGC Gly	CCG Pro	GGA Gly	CCC Pro	GGA Gly	CCC Pro	GGG Gly	CCC Pro	GGG Gly	CCC Pro	GGG Gly	1454
		450					455					460				
CCC Pro	GGC Gly	GCA Ala	GAC Asp	ATG Met	CAG Gln	CGC Arg	AGC Ser	TAT Tyr	GAC Asp	AGC Ser	TAC Tyr	TAT Tyr	TAC Tyr	CCC Pro	GCG Ala	1502
		465					470				475					
GCG Ala	GGG Gly	CCC Pro	GGA Gly	CCG Pro	CGG Arg	CGC Arg	GGG Gly	ACC Thr	TGC Cys	GCG Ala	CTC Leu	GGC Gly	GGC Gly	AGC Ser	CTG Leu	1550
	480					485				490						
GGC Gly	AGC Ser	CTG Leu	CCT Pro	GCC Ala	AGC Ser	CCC Pro	TTC Phe	CGC Arg	ATC Ile	CCC Pro	GAG Glu	GAC Asp	GAC Asp	GAG Glu	TAC Tyr	1598
495					500					505					510	

-95-

GAG ACC ACG CAG GAG TGC GCG CCC CCG CCG CCG CCG CCG CCG CCG CCG	1646
Glu Thr Thr Gln Glu Cys Ala Pro Pro Pro Pro Pro Pro Pro Pro Pro	
515 520 525	
CGC GGT GCG TCC CGC AGG ACG TCG GCG GGG CCC CCG CGC TGG CGC CGC	1694
Arg Gly Ala Ser Arg Arg Thr Ser Ala Gly Pro Arg Arg Trp Arg Arg	
530 535 540	
TCG CGC CTC AAC GGG CTG GCG GCG CAG CGC GCA CCG GCG GCG AGG GAC	1742
Ser Arg Leu Asn Gly Leu Ala Ala Gln Arg Ala Arg Ala Ala Arg Asp	
545 550 555	
TCG CTG TCG CTG AGC AGC GGC TCG GGC GGC GGC TCA GCC TCG GCG TCG	1790
Ser Leu Ser Leu Ser Ser Gly Ser Gly Gly Gly Ser Ala Ser Ala Ser	
560 565 570	
GAC GAC GAC GCG GAC GAC GCG GAC GGG GCG CTG GCG GCC GAG AGC ACA	1838
Asp Asp Asp Ala Asp Asp Ala Asp Gly Ala Leu Ala Ala Glu Ser Thr	
575 580 585 590	
CCT TTC CTG GGC CTG CGT GGG GCG CAC GAC GCG CTG CGC TCG GAC TCG	1886
Pro Phe Leu Gly Leu Arg Gly Ala His Asp Ala Leu Arg Ser Asp Ser	
595 600 605	
CCG CCA CTG TGC CCG GCG GCC GAC AGC AGG ACT TAC TAC TCA CTG GAC	1934
Pro Pro Leu Cys Pro Ala Ala Asp Ser Arg Thr Tyr Tyr Ser Leu Asp	
610 615 620	
AGC CAC AGC ACG CCG GCC AGC AGC AGA CAC AGC CCG GGG CCG CCC CCG	1982
Ser His Ser Thr Arg Ala Ser Ser Arg His Ser Arg Gly Pro Pro Pro	
625 630 635	
CGG GCC AAG CAG GAC TCG GCG CCA CTC TAGGGCCCCG CCGCGCGCCC CTCCGCC	2036
Arg Ala Lys Gln Asp Ser Ala Pro Leu	
640 645	
CCGCCCGCCC CACTATCTTT AAGGAGACCA GAGACCGCCT ACTGGAGAGA AAGGAGGAAA	2096
AAAGAAATAA AAATATTTTT ATTTTCTATA AAAGGAAAAA AGTATAACAA AATGTTTTAT	2156
TTTCATTTTA GCAAAAATTG TCTTATAATA CTAGCTAACG GCAAAGGCGT TTTTATAGGG	2216
AAACTATTTA TATGTAACAT CCTGATTAC AGCTTCGGAA AAAAAAAGA AA	2268

## (2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 647 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Met Ser Glu Ser Lys Arg Arg Gly Arg Gly Arg Gly Lys Lys His Pro	
1 5 10 15	
Glu Gly Arg Lys Arg Glu Arg Glu Pro Asp Pro Gly Glu Lys Ala Thr	
20 25 30	
Arg Pro Lys Leu Lys Lys Met Lys Ser Gln Thr Gly Gln Val Gly Glu	
35 40 45	
Lys Gln Ser Leu Lys Cys Glu Ala Ala Ala Gly Asn Pro Gln Pro Ser	
50 55 60	
Tyr Arg Trp Phe Lys Asp Gly Lys Glu Leu Asn Arg Ser Arg Asp Ile	
65 70 75 80	
Arg Ile Lys Tyr Gly Asn Gly Arg Lys Asn Ser Arg Leu Gln Phe Asn	
85 90 95	
Lys Val Lys Val Glu Asp Ala Gly Glu Tyr Val Cys Glu Ala Glu Asn	
100 105 110	

-96-

Ile Leu Gly Lys Asp Thr Val Arg Gly Arg Leu Tyr Val Asn Ser Val  
 115 120 125  
 Ser Thr Thr Leu Ser Ser Trp Ser Gly His Ala Arg Lys Cys Asn Glu  
 130 135 140  
 Thr Ala Lys Ser Tyr Cys Val Asn Gly Gly Val Cys Tyr Tyr Ile Glu  
 145 150 155 160  
 Gly Ile Asn Gln Leu Ser Cys Lys Cys Pro Asn Gly Phe Phe Gly Gln  
 165 170 175  
 Arg Cys Leu Glu Lys Leu Pro Leu Arg Leu Tyr Met Pro Asp Pro Lys  
 180 185 190  
 Gln Lys Ala Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly  
 195 200 205  
 Ile Cys Val Ala Leu Leu Val Val Gly Ile Val Cys Val Val Ala Tyr  
 210 215 220  
 Cys Lys Thr Lys Lys Gln Arg Lys Gln Met His Asn His Leu Arg Gln  
 225 230 235 240  
 Asn Met Cys Pro Ala His Gln Asn Arg Ser Leu Ala Asn Gly Pro Ser  
 245 250 255  
 His Pro Arg Leu Asp Pro Glu Glu Met Gln Met Ala Asp Tyr Ile Ser  
 260 265 270  
 Lys Asn Val Pro Ala Thr Asp His Val Ile Arg Arg Glu Thr Glu Thr  
 275 280 285  
 Thr Phe Ser Gly Ser His Ser Cys Ser Pro Ser His His Cys Ser Thr  
 290 295 300  
 Ala Thr Pro Thr Ser Thr His Arg His Glu Ser His Thr Trp Ser Leu  
 305 310 315 320  
 Glu Arg Ser Glu Ser Leu Thr Ser Asp Ser Gln Ser Gly Ile Met Leu  
 325 330 335  
 Ser Ser Val Gly Thr Ser Lys Cys Asn Ser Pro Ala Cys Val Glu Ala  
 340 345 350  
 Arg Ala Arg Arg Ala Ala Ala Tyr Asn Leu Glu Glu Arg Arg Arg Ala  
 355 360 365  
 Thr Ala Pro Pro Tyr His Asp Ser Val Asp Ser Leu Arg Asp Ser Pro  
 370 375 380  
 His Ser Glu Arg Tyr Val Ser Ala Leu Thr Thr Pro Ala Arg Leu Ser  
 385 390 395 400  
 Pro Val Asp Phe His Tyr Ser Leu Ala Thr Gln Val Pro Thr Phe Glu  
 405 410 415  
 Ile Thr Ser Pro Asn Ser Ala His Ala Val Ser Leu Pro Pro Ala Ala  
 420 425 430  
 Pro Ile Ser Tyr Arg Leu Ala Glu Gln Gln Pro Leu Leu Arg His Pro  
 435 440 445  
 Ala Pro Pro Gly Pro Gly Pro Gly Pro Gly Pro Gly Pro Gly  
 450 455 460  
 Ala Asp Met Gln Arg Ser Tyr Asp Ser Tyr Tyr Tyr Pro Ala Ala Gly  
 465 470 475 480  
 Pro Gly Pro Arg Arg Gly Thr Cys Ala Leu Gly Gly Ser Leu Gly Ser  
 485 490 495  
 Leu Pro Ala Ser Pro Phe Arg Ile Pro Glu Asp Asp Glu Tyr Glu Thr  
 500 505 510  
 Thr Gln Glu Cys Ala Pro Pro Pro Pro Pro Arg Pro Arg Ala Arg Gly  
 515 520 525  
 Ala Ser Arg Arg Thr Ser Ala Gly Pro Arg Arg Trp Arg Arg Ser Arg  
 530 535 540  
 Leu Asn Gly Leu Ala Ala Gln Arg Ala Arg Ala Ala Arg Asp Ser Leu  
 545 550 555 560  
 Ser Leu Ser Ser Gly Ser Gly Gly Gly Ser Ala Ser Ala Ser Asp Asp  
 565 570 575  
 Asp Ala Asp Asp Ala Asp Gly Ala Leu Ala Ala Glu Ser Thr Pro Phe  
 580 585 590  
 Leu Gly Leu Arg Gly Ala His Asp Ala Leu Arg Ser Asp Ser Pro Pro  
 595 600 605  
 Leu Cys Pro Ala Ala Asp Ser Arg Thr Tyr Tyr Ser Leu Asp Ser His  
 610 615 620  
 Ser Thr Arg Ala Ser Ser Arg His Ser Arg Gly Pro Pro Pro Arg Ala  
 625 630 635 640  
 Lys Gln Asp Ser Ala Pro Leu  
 645

(2) INFORMATION FOR SEQ ID NO:33:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Arg 1	Ile	Lys	Tyr	Gly 5	Asn	Gly	Arg	Lys	Asn 10	Ser	Arg	Leu	Gln	Phe 15	Asn
Lys	Val	Arg	Val 20	Glu	Asp	Ala	Gly	Glu 25	Tyr	Val	Cys	Glu	Ala 30	Glu	Asn
Ile	Leu	Gly 35	Lys	Asp	Thr	Val	Arg 40	Gly	Arg	Leu	His	Val 45	Asn	Ser	Val
Ser	Thr 50	Thr	Leu	Ser	Ser	Trp 55	Ser	Gly	His	Ala	Arg 60	Lys	Cys	Asn	Glu
Thr 65	Ala	Lys	Ser	Tyr	Cys 70	Val	Asn	Gly	Gly	Val 75	Cys	Tyr	Tyr	Ile	Glu 80
Gly	Ile	Asn	Gln	Leu 85	Ser	Cys	Lys	Cys	Pro 90	Asn	Gly	Phe	Phe	Gly 95	Gln
Arg	Cys	Leu 100	Glu	Lys	Leu	Pro	Leu	Arg 105	Leu	Tyr	Met	Pro	Asp 110	Pro	Lys
Gln	Ser	Val 115	Leu	Trp	Asp	Thr	Pro 120	Gly	Thr	Gly	Val	Ser 125	Ser	Ser	Gln
Trp 130	Ser	Thr	Ser	Pro	Ser	Thr 135	Leu	Asp	Leu	Asn					

- 98 -

What is claimed is:

1. An isolated nucleic acid encoding a Don-1 polypeptide.
2. An isolated nucleic acid of claim 1, wherein  
5 the nucleic acid encodes an amino acid sequence of SEQ ID NO:2, 4, 6, 8, or 32.
3. A nucleic acid of claim 1, wherein said nucleic acid encodes a soluble Don-1 polypeptide.
4. A nucleic acid of claim 1, wherein said  
10 nucleic acid comprises the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, or 31.
5. A nucleic acid of claim 1, wherein said nucleic acid encodes the epidermal growth factor (EGF) domain of Don-1 having SEQ ID NO:11.
- 15 6. A nucleic acid of claim 1, wherein said nucleic acid encodes the extracellular domain of Don-1.
7. A nucleic acid encoding a hybrid polypeptide, said hybrid polypeptide comprising a first portion and a second portion, said first portion comprising a Don-1  
20 polypeptide and said second portion comprising an immunoglobulin constant (Fc) region.
8. A nucleic acid of claim 7, wherein the first portion comprises the epidermal growth factor (EGF) domain of Don-1.
- 25 9. A nucleic acid of claim 1 encoding the amino acid sequence of the Ig domain of Don-1.



- 99 -

10. A nucleic acid of claim 1 encoding the amino acid sequence of the transmembrane (TM) domain of Don-1.

11. An isolated nucleic acid of claim 1 comprising the nucleotide sequence of the *don-1* gene  
5 contained in A.T.C.C. deposit 98096, 98097, or 98098.

12. An isolated nucleic acid of claim 1 that hybridizes to the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, or 31 or its complement.

13. An isolated nucleic acid of claim 12, wherein  
10 the nucleic acid encodes a polypeptide that activates receptor-type tyrosine kinases that have a molecular weight of about 185 kDa.

14. An isolated nucleic acid of claim 1 that hybridizes to the nucleotide sequence of the *don-1* gene  
15 contained in A.T.C.C. deposit 98096, 98097, or 98098.

15. An isolated nucleic acid of claim 14, wherein the nucleic acid encodes a polypeptide that activates receptor-type tyrosine kinases that have a molecular weight of about 185 kDa.

20 16. An isolated nucleic acid of claim 1 that hybridizes to the nucleotide sequence of the transmembrane (TM) domain of the *don-1* gene, wherein the isolated nucleic acid encodes a polypeptide that activates receptor-type tyrosine kinases that have a  
25 molecular weight of about 185 kDa.

17. An isolated nucleic acid of claim 1 that hybridizes to the nucleotide sequence of the epidermal growth factor (EGF) domain of the *don-1* gene, wherein the

- 100 -

isolated nucleic acid encodes a polypeptide that activates receptor-type tyrosine kinases that have a molecular weight of about 185 kDa.

18. A host cell comprising the nucleic acid of  
5 claim 1.

19. A nucleic acid vector comprising the nucleic acid of claim 1.

20. A nucleic acid vector of claim 19, wherein the vector is an expression vector.

10 21. A substantially pure Don-1 polypeptide.

22. A substantially pure polypeptide of claim 21, wherein said polypeptide is soluble.

23. A polypeptide of claim 21, wherein said polypeptide comprises the epidermal growth factor (EGF)  
15 domain of Don-1.

24. A polypeptide of claim 21, wherein said polypeptide comprises the extracellular domain of Don-1.

25. A polypeptide of claim 21, wherein said polypeptide comprises the amino acid sequence of SEQ ID  
20 NO:2, 4, 6, 8, or 32.

26. A polypeptide of claim 21, wherein said polypeptide is encoded by the nucleic acid sequence of SEQ ID NO:1, 3, 5, 7, or 31.

- 101 -

27. A polypeptide of claim 21, wherein said polypeptide is encoded by the *don-1* gene contained in A.T.C.C. deposit 98096, 98097, or 98098.

28. A substantially pure polypeptide of claim 21,  
5 wherein the polypeptide is at least 80% identical to the amino acid sequence of the epidermal growth factor (EGF) domain of Don-1.

29. The polypeptide of claim 28, wherein the EGF domain has the sequence of SEQ ID NO:11.

10 30. A substantially pure polypeptide of claim 1, wherein the polypeptide is at least 80% identical to the amino acid sequence of the Ig domain of Don-1.

31. The polypeptide of claim 30, wherein the Ig domain extends from about amino acid 16 to about amino  
15 acid 70 in SEQ ID NO:2, 4, or 6, or from about amino acid 54 to about amino acid 108 in SEQ ID NOs:8 and 32.

32. A substantially pure polypeptide of claim 1,  
wherein the polypeptide is at least 90% identical to the amino acid sequence of the transmembrane (TM) domain of  
20 Don-1.

33. The polypeptide of claim 32, wherein the TM domain has the sequence of SEQ ID NO:20.

34. A substantially pure polypeptide comprising a first portion and a second portion, said first portion  
25 comprising a Don-1 polypeptide and said second portion comprising an immunoglobulin constant (Fc) region or a detectable marker.

- 102 -

35. An antibody that specifically binds to a Don-1 polypeptide.

36. A pharmaceutical composition comprising a polypeptide of claim 21.

5           37. A method for detecting Don-1 in a sample, the method comprising:

          obtaining a biological sample;

          contacting the sample with an anti-Don-1 antibody of claim 35 under conditions that allow the formation of  
10 Don-1-antibody complexes; and

          detecting the complexes, if any, as an indication of the presence of Don-1 in the biological sample.

          38. A method for stimulating proliferation of a cell, the method comprising administering to the cell an  
15 amount of a Don-1 polypeptide effective to stimulate proliferation of the cell.

          39. A method for decreasing proliferation of a cell, the method comprising administering to the cell an  
amount of a Don-1 polypeptide inhibitor effective to  
20 decrease proliferation of the cell.

          40. A method of claim 39, wherein said inhibitor is an antibody that selectively binds to Don-1.

          41. A method of obtaining a splice variant cDNA of the *don-1* gene, the method comprising

25           obtaining a labeled probe comprising an isolated nucleic acid that encodes all or a portion of the epidermal growth factor (EGF) domain of Don-1;

          screening a nucleic acid fragment library with the labeled probe under conditions that allow hybridization

- 103 -

of the probe to nucleic acid fragments in the library to  
form nucleic acid duplexes;  
isolating labeled duplexes, if any; and  
preparing a full-length cDNA from the fragments in  
5 any labeled duplex to obtain a splice variant cDNA of the  
*don-1* gene.

42. A method of claim 41, wherein the EGF domain  
has the amino acid sequence of SEQ ID NO:11.

43. A method of obtaining a gene related to the  
10 *don-1* gene, the method comprising  
obtaining a labeled probe comprising an isolated  
nucleic acid that encodes all or a portion of the  
transmembrane (TM) domain of Don-1;  
screening a nucleic acid fragment library with the  
15 labeled probe under conditions that allow hybridization  
of the probe to nucleic acid fragments in the library to  
form nucleic acid duplexes;  
isolating labeled duplexes, if any; and  
preparing a full-length gene sequence from the  
20 nucleic acid fragments in any labeled duplex to obtain a  
gene related to the *don-1* gene.

44. A method of claim 43, wherein the TM domain  
has the amino acid sequence of SEQ ID NO:20.

Fig. 1

CCTAAGGGCAAAAACATCAAGAAAGAGGTGGGCAAGATCCTGTGGACTGACTGGCC	56
M X S Q T G E V G E K Q S L	14
CACCCGGGCTAAGCTGAAGAAG ATC AAG AGC CAG ACA GGA GAG GTG GGT GAG AAG CAG TCG CTC	120
K C E A A A G N P Q P S Y R W F K D G K	34
AAG TGT GAG GCA GCG GCG GGA AAC CCC CAG CCC TCC TAT CCC TGG TTC AAG GAT GGC AAG	180
E L N R S R D I R I K Y G N V R K N S R	54
GAA CTC AAC CGG AGT CGT GAT ATT CGC ATC AAG TAT GGC AAT GTC AGA AAG AAC TCA CGC	240
L Q F N K V R V E D A G E Y U C E A I N	74
CTA CAG TTC AAC AAA GTG AGG GTG GAG GAT GGC GCG GAG TAC GTC TGT GAG GCG GAG AAC	100
I L G K D T V R G R L K V N S V S T T L	94
ATC CTT GGG AAG GAC ACC GTG AGG GCG GGA CTC CAT GTC AAC AGC GTG AGC ACC ACT CTC	160
S S W S G H A R K C N E T A K S Y C V N	114
TCA TCC TCG TCG GGA CAT GCG CGG AAG TCC AAT CAG ACC GCG AAG TCC TAC TGT GTG AAT	420
G G V C Y Y I E G I N Q L S C K C P N G	134
GGA GCG GTG TCC TAC TAC ATC GAG GCG ATC AAC CAG CTC TCC TCC AAA TGT CCA AAC GGA	480
F P G Q R C L E K L P L R L Y M P D P K	154
TTC TTC GGA CAG AGA TGT TTG GAG AAA CTC CTT TTG GGA TTG TAC ATC CCA GAT CTT AAG	540
Q K A E E L Y Q K R V L T I T G I C V A	174
CAA AAG GCT GAG GAG CTG TAC CAG AAG AGA GTG CTC ACA ATT ACT GGT ATC TGT GTG GCG	600
L L V V G I V C V V A Y C K T K K G R R	194
CTG CTG GTC GCG ATC GTC TGT GTG GTC GCG TAC TGC AAG ACC AAA AAA CAG ACC AGG	660
Q M E H H L R Q N M C P A H Q N R S L A	214
CAG ATC CAT CAT CAT CTC CGG CAG AAC ATG TCC CCA GCG CAC CAG AAC CGA ACC CTG GCG	720
N G P S H P R L D P E E I Q M A D Y I S	234
AAC GGG CCC AGC CAC CTT CGG CTG GAC CTT GAG GAG ATC CAG ATG GCA GAT TAC ATC TCC	780
K N V P A T D H V I R R E A E T T F S G	254
AAA AAT GTG CCA CTT ACA GAC CAC GTG ATC CGG AGG GAA GGT GAG ACC ACC TTC TGT GCG	840
S H S C S P S H K C S T A T P T S S H R	274
AGC CAC TCC TGT TCA CTT TGT CAC CAC TCC TCC ACA GCG ACC GCG ACC TCC ACC CAC AGA	900
H E S H T W S L E R S E S L T S D S Q S	294
CAT GAG AGC CAC ACC TGG AGC CTG GAA CTT TCA GAG AGC CTG ACC TCG GAT TCC CAG TCA	960
G I M L S S V G T S K C N S P A C V E A	314
CGC ATC ATC CTA TCA TCA GTA GCG ACC AGC AAG TCC AAC AGC CCA GCA TGT GTG GAG GCA	1020
R A R R A A A Y S Q E E R R A A M P P	334
CGG GCG CGG AGG GCA GCA GCG TAC AGC CAG GAG GAG CGC AGC GGT GCG ATG CCA CCG	1080
Y H D S I D S L R D S P H S E R Y V S A	354
TAC CAT CAC TCC ATA GAC TCG CTG CGT GAC TGT CCA CAC AGT GAA AGG TAC CTG TCA CCG	1140

L	T	P	A	R	L	S	P	V	D	F	M	Y	S	L	A	T	Q	V	
TTG	ACC	ACC	CCC	GCT	CDC	CTC	TCC	CCC	GTC	GAC	TTT	CAC	TAC	TCG	CTG	GGC	AGG	CAG	GTC
374	1200																		
P	T	F	E	I	T	S	P	N	S	A	H	A	V	S	L	P	Z	A	A
CGG	ACT	TTT	GAG	ATC	ACG	TGC	CCC	AAC	TTT	GCC	CAT	GCC	GTC	TCG	CTG	CCG	CCC	GCC	CGC
394	1260																		
P	I	S	Y	R	L	A	E	Q	Q	P	L	L	R	H	P	A	P	P	G
CCC	ATC	ACC	TAC	CCC	CTG	GGG	GAG	CAG	CAG	CTC	CTC	CTG	GGG	CAT	CCA	GGC	CCG	CCC	GGC
414	1320																		
P	G	P	G	S	G	P	G	A	D	M	Q	R	S	Y	D	S	Y	Y	Y
CCG	GGG	CCG	GGG	TGG	GGG	CCC	GGA	GGC	GAC	ATG	CAG	CCC	AGC	TAC	GAC	AGC	TAC	TAC	TAC
434	1380																		
P	A	A	G	P	G	P	R	R	S	A	C	A	L	G	G	S	L	G	S
CTT	GGC	GGC	GGG	CCC	GGG	CCG	CCG	CCG	ACC	GCC	TGG	GGC	CTG	GGC	AGC	AGC	TTG	GGC	AGC
454	1440																		
L	P	A	S	P	F	R	I	P	E	D	D	E	Y	E	T	T	Q	E	C
CTG	CCC	GGC	AGC	CCC	TTT	CDC	ATC	CCG	GAG	GAC	GAG	GAG	TAC	GAG	ACC	ACG	CAG	GAG	TCG
474	1500																		
A	P	P	P	P	P	P	P	P	T	R	G	A	S	R	P	T	S	A	G
AGG	CCC	CCC	CCC	CCC	CCC	CCC	CCC	CCC	ACC	CCG	GGC	GGC	TCG	CCC	AGG	ACG	TCG	GGC	GGC
494	1560																		
P	R	R	W	R	R	S	R	L	N	G	L	A	A	Q	R	A	F	A	A
CCG	CCG	CCG	TGG	CCG	CCG	TCC	CCG	CTC	AAC	GGC	TTG	GGC	GGC	CAG	GGC	GCA	CCG	GGC	GGC
514	1620																		
R	D	S	L	S	L	S	S	G	S	G	C	G	S	A	S	A	S	D	D
CCG	GAC	TCC	CTG	TCA	TTG	AGC	AGC	GCT	TGG	GGC	TGG	GGC	TGG	GGC	TGG	GGC	TGG	GAC	GAC
534	1680																		
D	A	D	D	A	D	G	A	L	A	A	E	S	T	P	F	L	G	L	R
GAC	GGC	GAC	GAC	GGC	GAC	GGG	GGC	CTG	GGC	GGC	GAG	AGC	ACG	CCA	TTT	CTC	GGC	CTG	CCA
554	1740																		
A	A	H	D	A	L	R	S	D	S	P	P	L	C	P	A	A	D	S	R
GGG	GGC	CAC	GAC	GGC	TTG	CCG	TGG	GAC	TGG	CCG	CCG	CTG	TGG	CCG	GGC	GGC	GAC	AGC	AGG
574	1800																		
T	Y	Y	S	L	D	S	H	S	T	R	A	S	S	R	H	S	R	G	P
ACT	TAC	TAC	TCC	CTG	GAC	AGC	CAC	AGC	AGC	CCG	GGC	AGC	AGC	AGA	CAC	AGC	CCG	GGC	CCG
594	1860																		
P	T	R	A	K	Q	D	S	G	P	L									

[illegible]

Fig. 2

	CCTAACGGCCGCAAAAAACATCAAGAAAGAGAGTGGCGAAGATCTCTGTGCACTGACTCCGC	
	M K S Q T G E V G E K Q S L	56
CACCCGGCCCAAGCTGAAGAAG	ATG AAG ACC CAG ACA GGA CAG GTG GGT GAG AAG CAG TCG CTC	14
		120
K C E A A A G N P Q P S Y R W F X D G K		36
AAG TGT CAG GCA GCG GCG GGA AAC CCC CAG CCC TCG TAT CCG TGG TTC AAG GAT GGC AAG		180
E L N R S R D I R I K Y G N V R K H S R		54
GAA CTC AAC CCG AGT CGT GAT ATT CCG ATC AAG TAT GCG AAT GTC AGA AAG AAC TCA CCG		240
L Q F N K V R V E D A G E Y V C E A E N		74
CTA CAG TTC AAC AAA GTG AGG GTG GAG GAT GCG GCG GAG TAC CTC TGT GAG GCG GAG AAC		300
I L G K D T V R G R L H V N S V S T T L		94
ATC CTT GGG AAG GAC ACC GTG AGG GGC CGA CTC CAT GTC AAC ACC GTG AGC ACT ACT CTC		360
S S W S G H A R K C N E T A K S Y C V N		114
TCA TCC TCG TCG GGA CAT GCG CCG AAG TCC AAT GAG ACC GCG AAG TCC TAC TGT GTG AAT		420
G G V C Y Y Z E G I N Q L S C R C P N G		134
CGA GCG GTC TGC TAC TAC ATC GAG GGC ATC AAC CAG CTC TCC TGC AAA TGT CCA AAC GGA		480
F F G Q R C L E X L P L R L Y H P D P K		154
TTT TTC GGA CAG AGA TGT TTG GAG AAA CTC CTT TTG CGA TTT TAC ATG CCA GAT CTT AAG		540
Q S V L W D T P G T G V S S S Q W S T S		174
CAA ACT GTC CTG TGG GAT ACA CCG GGG ACA GGT GTC AGC AGT TCG CAA TGG TCA ACT TCT		600
P S T L D L N -	(SEQ ID No:19)	181
CCA AGC ACC TTG GAT TTG AAT TGA		624
AGCAGCCTGAGGAGCTGTATCCAGAAGAGAGTCTGTGACAATTACTGGTATCTGTGTGGCCCTGTCTGGTCTGGGCATCTT		
CTGTGTCTGTCTGGCTACTGTCAAGACCAAAAAACAGAGGAGCCAGATGCAATCAATCTCTGGCAGAACATGTGCCCCAGCC		
CAGCAGAACCCGAAGCTCTGGCCAAAGGGGCCAGCCACCCCTGGCTTGCAGCTTGAGGAGATCCAGATGGCAGATTACATCT		
CCAAAAATGTGCCAGCTACAGACCACTGATCTGGAGGGGAGGCTGAGACCACTTCTGTGGGAGCCACTCTCTTTTCAAC		
TTCTCACCACCTCTCTCCACGCCACGCCCCACTCTTCAGCCACAGACATGAGAGCCACAGCTGGAGCCCTGGAACTTCAGAG		
AGCCTGACCTCGGATTTCCCACTCAGGCAATCTCTATCATCATAGTAGGCCACAGCAAGTGCAACAGCCAGCAGATGTGTGG		
AGGCACGGGGCGGAGGGGAGCAGCCTTACAGCCAGGAGGAGCGGGCGAGGGCTGCCATGCCACCTTACCATGACTGCAT		
AGACTCTCTCGCTGACTCTCTCCACACAGTGAAAGGTAGCTGTTCAGCCTTGACCACGCCCTGGCTGGCTCTGGCCGTGGAC		
TTCCACTACTCTGCTGGCCAGCCAGGTGCCGACTTTTCAGATTCAGCTGGCCACCTTGTGGCATGCTCTGCTTCTGCTT		
CCCCCGCCGCCCAATAGCTATCCGCTCTGGCCAGCAGCCAGCCCTCTCTGGCATTCAGCGCTCTCTCTCTCTCTCTCTCTCTCT		
GCTGGGGCCCGGAGCCGACATGACGCCAGCTAGCAGCAGCTACTACTACTCTCTGGCCCGGGCCCGCCCGCCCGCCCGCC		
GCTTGGCCCTCTGGGAGCCAGCTTGGGAGGCTTGGCCAGGCTTCTGGCATTCCTGGAGGAGCAGCAGGATAGCAGAGCTA		
CCGACAGTTCGGGCT		
(SEQ ID No:2)		



CAGCTACAGCGGACAGCAGCCAGCACGGCAGGCAGGCCAGCAGCAGCAG	60
CAGCGAGAGCGGGCAGCAGCAGCAGGAGCAGCAGCAACAACAGCAGCATCTCTCGTCCCC	120
TGCCCCCCAGAGCCCCTGGCCGAJACCAGCCCGCAGCCCCGAGCCCCGAGCEGGAG	180
AGCCCCCGCCCGTTTCGCGAGCCGAGCCGCGCGGGCATGAGCGCGGACCGCCCGCCG	240
CTTCTCCAATGCTGTCTTTCGGTGTTGCTCGCTCGCTGTACTCGCCCGAGCCTCAAATCAGT	300
GCAGGACCAGCGGTACAAGGCACCCGTGGTGGTGGAGGGCAAGGTACAGGGGGTGGTCC	360
AGCCCCCGGCTCCAGCTCCAACAGCACCCCGAGAGCCCCCGCCCTCGCGTGGGTGGCGTT	420
GGTAAAAGTGTCTGGACAAGTGGCCGCTCCGGAGCGGGGGGGCTGCAGCGCCAGCAGGTGAT	480
CAGCGTGGGCTCTCTGTGTGCCGCTCGAAAGGAAC CAGCGCTACATTTTTTCTGTGAGCC	540
CACCGAACAGCCCTTAGTCTTTTAAGACGGCGTTTGCCCGCCGTGATACCAACGGCAAAAA	600
CTCAAGAATAAGGTGGSCAAGATCTCTOTCAGTCTGCTCGCCACCCCGCCCAAGTTGAAG	660
M K S Q T G Q V G E R O S L K C E A A AAGATTGAAGAGCCAGACGGGACAGGTGGGTGAGAAAGCAATCGCTGAAGTGTGAGGCAGCA	720
A G N P Q P S Y R W F X D G K E L N R S GCCCGTAATCCCCAGCCTTCTGTACCGTTGGTTCAAGGATGGCAAGGAGCTCAACCGCAGC	780
R D I R I K Y G N G R K N S R L O P N K CGAGACATTEGCAATCAATATGSCAACGGCAGAAAGAACTCAGGACTACAGTTCAACAG	840
V K V E D A G E Y V C E A E N I L G K D GTGAAGGTGGAGGACGCTGGGGAGTATGTCTCGGAGGCCGAGAACATCTCTGGGGAAGGAC	900
T V E G R L Y V N S V S T T L S S W S G ACCGTCCCGGGCGCCGCTTTACGTCAACAGCGTGAGCACCCAGCTGTCTCTGCTGGGGG	960
H A R K C N E T A K S Y C V N G G V C Y CAGCCCCGGAAGTGCAACGAGACAGCCAGTGCTATTGGCTCAATGGAGCGGCTCTGCTAC	1020
Y I E G I N Q L S C K C P N G F F G Q R TACATCGAGCGCATCAACCAAGCTCTCTCTGCAATGTCCAAATGGATTCTCTGGGACAGAGA <sup>194</sup>	1080
C L E K L P L R L Y M P D P K Q K H L G TGTTTGGAGAAACTGCCCTTTGGGATGTGTACATGCCAGATCTTAAGCAAAAGCAGCTTGG	1140
F E L K E A E E L Y Q K R V L T I T G I TTTGAATTAAAGCAAGCCCGAGGAGCTGTATCCAGAGAGGGTCTTGACCATCAGCGGCATC	1200
C V A L L V V G I V C V V A Y C K T K K TGGTGCGCTCTCTGTGGTCTGGGCATCGCTCTGTGTGGTGGCCCTACTGCAAGACCAAAAA	1260
Q R K Q M R N K L R Q N M C P A H Q N R CAGCGGAAGCAGATGCACAACCACTCTCCGCAGAAATGTGCCCGGCCCATCAGAAACCG	1320
S L A N G P S H P R L D P E E I Q M A D AGCTTGGCGCAATGGCGCCAGCCACCCCGCTGGACECAGAGGAGATCCAGATGCCAGAT	1380

FIGURE 3 (continued)

Y I S K N V P A T D H V I R R E T E T T 1440  
TATATTTCCAAGAACGTGCCAGCCACAGACCATGTCTCAGGAGAGAACTGAGACCACC  
F S G S H S C S P S H H C S T A T P T S 1500  
TTCTCTGGGAGCCACTCCTGTTCTCCTTCTCACCAGCTGCTCCACAGCCACAGCCACCTCC  
S H R H E S H T W S L E R S E S L T S D 1560  
AGCCACAGACACGAGAGCCACACGTGGAGCCTGGAAACGTTCTGAGAGCCTGACTTCTGAC  
S Q S G I M L S S V G T S K C N S P A C 1620  
TCCCAGTCGGGGATCATGCTATCATCAGTGGGTACCAGCAAAATGCAACAGCCAGCATGT  
V E A R A R R A A A Y N L E E R R R A T 1680  
GTGGAGCCCCCGGCAAGCCGGCCAGCAGCCTACAACCTGGAGGAGCCGGCCAGGGCCACC  
A P P Y H D S V D S L R D S P R S E R Y 1740  
GGCCACCCCTATCAGGATTCGGTGGACTCCTTCGGGACTCCCCACACAGCGAGAGGTAC  
V S A L T T P A R L S P V D F H Y S L A 1800  
GTCTGGCCCTGACCACGCCCGCCGCTCTCGCCCGTGGACTTCGACTACTCGCTGGCC  
T Q V P T F E I T S P N S A H A V S L P 1860  
ACGCAGGTGCCAACTTTCGAGATCACTGCCCCCAACTGGGCCACGCCCTGTCTGCTGCCG  
P A A P I S Y R (SER in AB:6)  
CCGGCGCGCCCATCAGTTACCGC 1884 (SER in AB:5)

Fig. 4

CGGGCGGGCGGGCGGAGCGCGGAGCGGAGAGCTGAGGCGCTCCACCGCTGGGACCC 60

M S E S R R R G R G R G K K H P E  
CGTGCAGAA TGTGGAGTCCAGGAGGAGGGCGGGCGGGCGGCAAGAAGCACCAGAGG 120

G R K R E R E P D P G E K A T R P K L K  
GGAGGAAGCGGGAGAGGGAGCCCGATCCCGGGGAGAAAGCCACCGGGCCAAAGTTGAAGA 180

K M K S Q T G Q V G E K Q S L K C E A A  
AGATGAAGAGCCAGACGGGACAGGTGGGTGAGAAGCAATCGCTGAAGTGTGAGGCAGCAG 240

A G N P Q P S Y R W F K D G K E L N R S  
CCGGTAATCCCCAGCCTTCTTACCGTTGGTTCAAGGATGGCAAGGAGCTCAACCGCAGCC 300

R D I R I K Y G N G R K N S R L Q P N K  
GAGACATTCGCATCAAAATATGGCAACGGCAGAAAGAACTCAGGACTACAGTTCAACAAGG 360

V K V E D A G E Y V C E A E N I L G K D  
TGAAGGTGGAGGACGCTGGGGAGTATGTCTCGGAGGGCGAGAACA TCTGGGGAAGGACA 420

T V R G R L Y V N S V S T T L S S W S G  
CCGTCCGGGGCGGGCTTTACGTCAACAGCGTGAGCACCACCTGTTCATCTCTGGTCCGGGC 480

H A R K C N E T A K S Y C V N G G V C Y  
ACGCCCGGAAGTCCAAAGGAGACAGCCAGTCTTATTCGGTCAATGGAGGCGTCTGCTACT 540

Y I E G I N O L S C K C P N G F P G Q R  
ACATCGAGGGCATCAACAGCTCTCTCTGCAATGTCCAAATGGA TCTTCGGCAGAGAT 600

C L E K L P L R L Y M P D P K Q K A E E  
GTTTGAGAAACTGCGCTTTGGGATTGTACATGCCAGATCTTAAGCAAAAAGCCGAGGAGC 660

L Y Q K R V L T I T G I C V A L L V V G  
TGTAACGAGAGAGGGTCTCTGACCATCACGGGCAATCTCGGTGGCTCTGCTGGTCTGGGCA 720

I V C V V A Y C K T K K Q R K Q M H N H  
TCTCTGTGTGGTGGCTTACTGCAAGACCAAAAACAGCGGAAGCAGATGCACAACACC 780

L R Q N M C P A H Q N R S L A N G P S H  
TCCGGCAGAAATGTGCCCCGGCCATCAGAACCGGAGCTTGGCCAAATGGGCCCAGGCCAC 840

P R L D P E E I Q M A D Y I S K N V P A  
CCCGGCTGGACCCAGAGGAGATCCAGATGGCAGATTATATTTCCAAGAACGTGCCAGCCA 900

T D H V I R R E T E T T F S G S H S C S  
CAGACCATGTCTATCAGGAGAGAACTGAGACCACTTCTCTGGGAGCCACTCTCTTCTCT 960

P S H H C S T A T P T S S H R H E S H T  
CTTCTCAGCACTGCTCCACAGCCACACCCACCTCCAGCCACAGACACGAGAGCCACAGCT 1020

W S L E R S E S L T S D S Q S G I M L S  
GGAGCCTGGAAAGTTCTGAGAGCCTGACTTCTGACTCCAGTCCGGGATCATGCTATCAT 1080

S V G T S K C N S P A C V E A R A R R A  
CAGTGGGTACCAGCAAA TCCAACAGCCAGCAATGTGTGGAGGGCCCGGCAAGCCGGGCAG 1140

A A Y N L E E R R R R A T A P P P Y H D S V	
CAGCCTACAACTTGGAGGAGCGCGCGCAGGGCCACCGCGCCACCCCTATACAGATTCCGTGG	1200
D S L R D S P H S E R Y Y V S A L T T P A	
ACTCCCTTCGGGACTCCCCACACAGCGAGAGGTACGTTGTCGGCCCTGACACAGCGCCCGCGC	1260
R L S . P V D F H Y S L A T Q V P T F E I	
GCCTTCGCCCGTGGACTTCCACTACTCGCTGGCCACCGCAGGTGCCAACTTTTCGAGATCA	1320
T S P N S A H A V S L P P C A A P I S Y R	
CGTCCCCCACTCGGCGCAGCGCGTGTCTGCTGCCCGCGCGGCCCATCAGTTACCGCC	1380
L A E Q Q P L L R H P A P P G P G P G P	
TGGCCGAGCAGCAGCCCTTACTCGGGCACCGGCGCCCCCGGCGCGGAGCCCGACCCG	1440
G P G P G P G A D T G I	
GGCCCCGGGCGCGGGCCCCGGCGCAGACACCGGAATTC	1476

(SEQ ID NO: 8)  
(SEQ ID NO: 7)

```

1
50
(SEQ ID NO: 9) ndf MIERKEGRGK GKGKCKGRCS RKGKPAEGD PFPALPPRLK EKKSQESAAG
(SEQ ID NO: 10) hfg-b MIERKEGRGK GKGKCKGRCS GKGKPAAGS QFPALPPRLK EKKSQESAAG
SEQ ID NO: 8 MIERKRGK. GKGKCKPGR KREREPDP... GKATAPRLK EKKSQTGGVG
SEQ ID NO: 6 ..... HKKSQTGGVG
SEQ ID NO: 2 ..... HKKSQTGGVG
SEQ ID NO: 4 .....

51
100
ndf SKLVLRCTTS SEYSSSLRFX FONGWELNRK NKPENTAIQK KPGKSELRLN
hfg-b SKLVLRCTTS SEYSSSLRFX FONGWELNRK NKPENTAIQK KPGKSELRLN
EKQSLKCEAA AGNPQPSYRW FKDGKELNR. SRDIRIKYGN GRKNSRLQFN
EKQSLKCEAA AGNPQPSYRW FKDGKELNR. SRDIRIKYGN GRKNSRLQFN
EKQSLKCEAA AGNPQPSYRW FKDGKELNR. SRDIRIKYGN GRKNSRLQFN
..... RIKYGN GRKNSRLQFN

101
150
ndf KASLADSGEY MCKVISKLGN DSASANITTV ESNEITGMP ASTETAYVSS
hfg-b KASLADSGEY MCKVISKLGN DSASANITTV ESNEITGMP ASTETAYVSS
KVKVEDAGEY VCEAENILGX DTVRGRLV. ....
KVKVEDAGEY VCEAENILGX DTVRGRLV. ....
KVKVEDAGEY VCEAENILGX DTVRGRLV. ....
KVKVEDAGEY VCEAENILGX DTVRGRLV. ....

151
200
ndf ESPRISVST EGANTSSSTS TSTTGTSHLI KCAEKETTC VNGGCTTVK
hfg-b ESPRISVST EGANTSSSTS TSTTGTSHLV KCAEKETTC VNGGCTTVK
.....NSVST .....TLSSWS .....GHAR KCMETAKSYC VNGGVCYYIE
.....NSVST .....TLSSWS .....GHAR KCMETAKSYC VNGGVCYYIE
.....NSVST .....TLSSWS .....GHAR KCMETAKSYC VNGGVCYYIE
.....NSVST .....TLSSWS .....GHAR KCMETAKSYC VNGGVCYYIE

201
250
ndf DLSNPSRYLC KCPNGFTGAR CTENV..... FMKVQ..... TOEXAZELY
hfg-b DLSNPSRYLC KCPNEFTGAR CONVMASFY KHLGIE..... FME.AEELY
GINQLS...C KCPNGFTGAR CLKLPLRLY MPDPKQK... ..AEELY
GINQLS...C KCPNGFTGAR CLKLPLRLY MPDPKQK... FELKEAEELY
GINQLS...C KCPNGFTGAR CLKLPLRLY MPDPKQK... ..AEELY
GINQLS...C KCPNGFTGAR CLKLPLRLY MPDPKQSVL.....

251
300
ndf QKQ/LTITGI CIALLVGIV CVVAYCKTKK QRKLEHRLR QSLRSEKNTL
hfg-b QKQ/LTITGI CIALLVGIV CVVAYCKTKK QRKLEHRLR QSLRSEKNTL
QKQ/LTITGI CIALLVGIV CVVAYCKTKK QRKQMHRLR QNMCFAMQN.
QKQ/LTITGI CIALLVGIV CVVAYCKTKK QRKQMHRLR QNMCFAMQN.
QKQ/LTITGI CIALLVGIV CVVAYCKTKK QRKQMHRLR QNMCFAMQN.
QKQ/LTITGI CIALLVGIV CVVAYCKTKK QRKQMHRLR QNMCFAMQN.
...WDTPTGV SSSQWSTSPS TLDLN*

```

SEQ ID NO: 20

Fig. 5

## FIGURES (continued)

```

101                                     150
ndf  VNIANGPHHP NPPFENVQLV NQYVSKNVIS SEHIVEREVE TSFSTSHYTS
hrq-b VNIANGPHHP NPPFENVQLV NQYVSKNVIS SEHIVEREAE TSFSTSHYTS
SEQ ID NO:8 RSLANGPSHP RLDPEEIQH. ADYISKNVPA TDHVIARETE TTFSGSHSCS
SEQ ID NO:6 RSLANGPSHP RLDPEEIQH. ADYISKNVPA TDHVIARETE TTFSGSHSCS
SEQ ID NO:2 RSLANGPSHP RLDPEEIQH. ADYISKNVPA TDHVIAREAE TTFSGSHSCS
SEQ ID NO:4 .....

351                                     400
ndf  TARKSTTVTQ TP.....SHS WSNCHTESVI SESNSVIRMS SVENSRRSSP
hrq-b TARKSTTVTQ TP.....SHS WSNCHTESIL SESNSVIRMS SVENSRRSSP
PSHKCSTATP TSSSRHESHT WSLERSESLT SDSQSGIMLS SVGTSKCNWP
PSHKCSTATP TSSSRHESHT WSLERSESLT SDSQSGIMLS SVGTSKCNWP
PSHKCSTATP TSSSRHESHT WSLERSESLT SDSQSGIMLS SVGTSKCNWP
.....

401                                     450
ndf  A..GGPRGRL RGLGGPRD.N SFLRHARETP DSYRDSPHSE R*.....
hrq-b T..GGPRGRL NGTGGPRECN SFLRHARETP DSYRDSPHSE RYVSALTTPA
ACVEARARRA AAYNLEERRR ATAPPYHDSV DSLRDSPHSE RYVSALTTPA
ACVEARARRA AAYNLEERRR ATAPPYHDSV DSLRDSPHSE RYVSALTTPA
ACVEARARRA AAYSQERRR AAPPYHDSI DSLRDSPHSE RYVSALTTPA
.....

451                                     500
ndf  .....
hrq-b RNSPVDHNP SPKSPPEM SPPVSEHVS MFSMAVSFFM EERPLLLVT
RLSPVDHNPYS LATQVPTFEI TSPNSAHAVS LPPAAPISYR LAEQQLLRH
RLSPVDHNPYS LATQVPTFEI TSPNSAHAVS LPPAAPISYR .....
RLSPVDHNPYS LATQVPTFEI TSPNSAHAVS LPPAAPISYR LAEQQLLRH
.....

501                                     550
ndf  .....
hrq-b PFRLEKCKTD KNPQQFSFY KNPAGKSNL PASPLRIVED EYETIQEYE
PAZPGPGPGP GPGPGGADT GI.....
PAPPGPGPGS GPGADHQRSY DSYTTPAAGP GPRRSACALG GSLGSLPASP
.....

551                                     600
ndf  .....
hrq-o PAQETVIGLA NSRAJORTCP NCHIANULEV DSNTSSQSSN SESETERDV
.....
FRIFEDDEYE TQECAPPPP PRPRTRGASH RTSAGPRWR RSLRLGLAAQ
.....

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10/13

FIGURE 5 (CONTINUED)

	601				650
ndf	.....	.....	.....	.....	.....
hry-b	GEDTFFLGIO	NPLAASLEAT	PAFRLADSR	NPAGRFSTOE	ZIQARLSSVT
SEQ ID NO: 8	.....	.....	.....	.....	.....
SEQ ID NO: 6	.....	.....	.....	.....	.....
SEQ ID NO: 2	RARAARDSL	LSSGSGCGSA	SASDDADDA	DGALAAESTP	FLGLRAAKDA
SEQ ID NO: 4	.....	.....	.....	.....	.....
	651				696
ndf	.....	.....	.....	.....	.....
hry-b	ANQDPPIAV*	.....	.....	.....	.....
	.....	.....	.....	.....	.....
	.....	.....	.....	.....	.....
	LRSDSPPLCP	AADSRYYSL	DSMSTRASSR	HSRGPPTRAK	QDSGFL
	.....	.....	.....	.....	.....

don-1	GHARRNETAKSYVNGGVVYIEGINQLS...KCPNGFFGQRLEKL P	Seq. 4
hrg-a	SHLVRAEKERTFVNGGECFMVKDLSNPSRYLCKCPGFTGARCTENV?	11
ndf	SHLIQAEEKERTFVNGGECFTVKDLSNPSRYLCKCPGFTGARCTENV?	12
hrg-b	SHLVRAEKERTFVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCONYVM	13
aria	SHLTQDIKQKAFVNGGECFMVKDLPNPPRYLCKCPNEFTGDRCONYVM	14
human hb-egf	GRRDHQLRKYKDFCIH.GECKYVKELRAPS...CIHPGYHGERCHGLSL	15
human egf	NSDSEQLSHDGYCLHDGVCMYIEALDKYA...CNCVVGYYIGERCQYRDL	16
amphiregulin	KKKNFQNAEFQNFCIH.GECKYIELEAVT...CKCQOEYFGERCEKSY	17
TGF- $\alpha$	SHFNDLPDSHTQFCH.GICRFLVQEDKPA...CMCHSGYVGARCEHADL	18

Fig. 6



																				M	S	
CCCCCCCCCCCCCAGCGGGGCGAGCGGAGAGCTGAGCGCCCTCCACCGCCTGGGACCCCTTCGAGA																				ATG	TGG	
E	S	R	R	R	G	R	G	R	G	K	K	H	P	E	G	R	K	R	E	22		
GAG	TCC	AGG	AGG	AGG	GGC	CGC	GGC	CGC	GGC	AAG	AAG	CAC	CCA	GAG	GGG	AGG	AAG	CGG	GAG	134		
R	E	P	D	P	G	E	K	A	A	T	R	P	K	L	K	K	M	K	Q	42		
AGG	GAG	CCC	GAT	CCC	GGG	GAG	AAA	GCC	ATC	CGG	CCC	AAG	TTG	AAG	AAG	ATG	AAG	AGC	GAG	194		
T	G	Q	V	G	E	K	Q	S	L	K	C	E	A	A	A	G	N	P	Q	62		
ACG	GGA	CAG	GTG	GGT	GAG	AAG	CAA	TCG	CTG	AAG	TGT	GAG	GCA	GCA	CCC	GGT	AAT	CCC	CAG	254		
P	S	Y	R	W	F	K	D	G	K	E	L	N	R	S	R	D	I	R	I	82		
CCT	TCC	TAC	CGT	TGG	TTC	AAG	GAT	GGC	AAG	GAG	CTC	AAC	CGC	AGC	CGA	GAC	ATT	CGC	ATC	314		
K	Y	G	N	G	R	K	N	S	R	L	Q	F	N	K	V	K	V	E	D	102		
AAA	TAT	GGC	AAC	GGC	AGA	AAG	AAC	TCA	CGA	CTA	CAG	TTC	AAC	AAG	GTG	AAG	GTG	GAG	GAC	374		
A	G	E	Y	V	C	E	A	E	N	I	L	G	K	D	T	V	R	G	R	122		
GCT	GGG	GAG	TAT	GTC	TGC	GAG	GCC	GAG	AAC	ATC	CTG	GGG	AAG	GAC	ACC	GTC	CGG	GGC	CGG	434		
L	Y	V	N	S	V	S	T	T	L	S	S	W	S	G	H	A	R	K	C	142		
CTT	TAC	TGC	AAC	AGC	GTG	AGC	ACC	ACC	CTG	TCA	TCC	TGG	TGG	GGG	CAC	GCC	CGG	AAG	TCC	494		
N	E	T	A	K	S	Y	C	V	N	G	G	V	C	Y	Y	I	E	G	I	162		
AAC	GAG	ACA	GCC	AAG	TCC	TAT	TGC	GTC	AAT	GGA	GGC	GTC	TCC	TAC	ATC	ATC	GAG	GGC	ATC	554		
N	Q	L	S	C	K	C	P	N	G	F	F	G	Q	R	C	L	E	K	L	182		
AAC	CAG	CTC	TCC	TGC	AAA	TGT	CCA	AAT	GGA	TTC	TTC	GGA	CAG	AGA	TGT	TTG	GAG	AAA	CTG	614		
P	L	R	L	Y	M	P	D	P	K	Q	K	A	E	E	L	Y	Q	K	R	202		
CCT	TTG	CGA	TTG	TAC	ATG	CCA	GAT	CCT	AAG	CAA	AAA	GCC	GAG	GAG	CTG	TAC	CAG	AAG	AGG	674		
V	L	T	I	T	G	I	C	V	A	L	L	V	V	G	I	V	C	V	V	222		
GTC	CTG	ACC	ATC	ACG	GGC	ATC	TGC	GTG	GCT	CTG	CTG	GTC	GTG	GGC	ATC	GTC	TGT	GTG	GTG	734		
A	Y	C	K	T	K	K	Q	R	K	Q	M	H	N	H	L	R	Q	N	M	242		
GCC	TAC	TGC	AAG	ACC	AAA	AAA	CAG	CGG	AAG	CAG	ATG	CAC	AAC	CAC	CTC	CGG	CAG	AAC	ATG	794		
C	P	A	H	Q	N	R	S	L	A	N	G	P	S	H	P	R	L	D	P	262		
TCC	CGC	GGC	CAT	CAG	AAC	CGG	AGC	TTG	GCC	AAT	GGG	CCC	AGC	CAC	CCC	CGG	CTG	GAC	CCA	854		
E	E	I	Q	M	A	D	Y	I	S	K	N	V	P	A	T	D	H	V	I	282		
GAG	GAG	ATC	CAG	ATG	GCA	GAT	TAT	ATT	TCC	AAG	AAC	GTG	CCA	GCC	ACA	GAC	CAT	GTC	ATC	914		
R	R	E	T	E	T	T	F	S	G	S	H	S	C	S	P	S	H	H	C	302		
AGG	AGA	GAA	ACT	GAG	ACC	ACC	TTC	TCT	GGG	AGC	CAC	TCC	TGT	TCT	CCT	TCT	CAC	CAC	TCC	974		
S	T	A	T	P	T	S	S															

[illegible]

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/14585

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 252.3, 320.1, 325; 436/94, 501; 514/2; 530/324, 350, 387.1; 536/23.5, 24.31; 935/1, 9, 11, 76, 77, 78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Nierman et al., eds., ATCC/NIH Repository Catalogue of Human and Mouse DNA Probes and Libraries, 1994, pages 1-70; see pages 1-58.	1-20
X	WO 94/08007 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 14 April 1994 (14.04.94), see entire document.	1-3, 5-10, 14-20
X	Stratagene Cloning Systems catalogue, 1994, pages 304-305.	12
A	HARDIE et al., The Protein Kinase FactsBook, " 1995, pages 165-167.	1-44

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

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Date of the actual completion of the international search

20 SEPTEMBER 1997

Date of mailing of the international search report

23 DEC 1997

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/14585

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CULOUSCOU et al., "HER4 Receptor Activation and Phosphorylation of the She Proteins by Recombinant Hergultin-Fe Fusion Proteins," The Journal of Biological Chemistry. 26 May 1995, Vol. 270, No. 21 pages 12857-12863; see the entire document.	1-44
A	WEN et al., "Neu. Differentiation Factor: A Transmembrane Glycoprotein Containing an EGF Domain and an Immunoglobulin Homology Unit," Cell. Vol. 69, 01 May 1992, pages 559-572.	1-44
A	TZAHAR et al., "ErbB-3 and ErbB-4 Function as the Respective Low and High Affinity Receptors of All Neu Differentiation Factor/Heregulin Isoforms," The Journal of Biological Chemistry. 07 October 1994, Vol. 269, Number 40, pages 25226-25233.	1-44
A	SLAMON et al., "Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER-2/neu Oncogene," Science. 09 January 1987, volume 235, pages 177-182.	1-44

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JS97/14585

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07H 21/04; C07K 1/00, 16/00; A61K 38/00; G01N 33/00, 33/566; A01N 37/18

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/320.1, 325; 436/94, 501; 514/2; 530/324, 350, 387.1; 536/23.5